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GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS BEARING²
ON HEREDITY AND VARIATION

VOLUME 4 1919

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CORRIGENDA

- Page 110, heading of third column of figure 11, for "64" read "35" days,
and for "22" read "18" generations.
- Page 110, heading of fourth column of figure 11, for "35" read "23" days,
and for "18" read "15" generations.
- Page 146, line 10, section "5" should be numbered "4".
- Page 146, line 9 from below, section "6" should be numbered "5".
- Page 147, line 22, section "7" should be numbered "6."
- Page 210, line 29, for " D'_s " read " D'_s "

CORRIGENDA (Continued)

Page 210, line 30, for " e_s " read " e^s ".

Page 215, last line of table 6, under 3 and 4, of single crossovers, for "29" and "436", read "22" and "443".

Page 236, first section of table 8, number of non-crossovers, for “4664” read “4644”, and in last section of table 8, total for “8167” read “8147”.

Page 242, insert, table A, reference number 1350, under heading 4, 6, for “. .” read “1 2”; reference number 1351, under heading 1, 6, for “. 1” read “. 2”; and under heading 4, 6, for “1 2” read “. .”; reference number 1368, total, for “243” read “241”; reference number 1369, under heading 1, 3, for “. .” read “. 1”; reference number 1415, under heading 3, 6, for “1 .” read “. .”; and under heading 4, 6, for “1 .” read “. .”; reference number 1417, under heading 3, 6, for “. .” read “1 .”, and under heading 4, 6, for “. .” read “1 .”; reference number 1426, under heading 2, 3, 6, for “. 2” read “. .”; reference number 1427, under heading 2, 3, 6, for “. .” read “. 1”; reference number 1429, under heading 3, 6, for “. .” read “. 2”.

Page 243, table D, heading, for " $\circ \frac{s e^s r_o}{D'}$ " read " $\circ \frac{s_e s_s e^s r_o}{D'}$."

Page 243, table D, reference number 740, under heading 4, for “26 28” read “26 18”; reference number 754, number headings 1, 3 to 3, 4, inclusive, for “| . . | . . | . . | 1” read “| 1 . | 1 1 | 1 . | 1 . | . 2 |”, and total, for “45” read “165”; reference number 756, under headings 1, 3 to 3, 4, inclusive, for “| 1 . | 1 1 | 1 . | 1 . | . 2 |” read “| . . | . . | . . | . 1 |”, and under total, for “238” read “118”; reference number 763, under heading 2, 3, for “1 .” read “: .”, and under total, for “171” read “172”; reference number 791, under total, for “164” read “172”; reference number 905, under heading 1, 3, for “2 3” read “2 .”, and under total, for “212” read “209”.

Page 244, table D, reference number 934, for data under headings, o to 2, 4, inclusive, substitute “| 19 17 | 1 4 | . 3 | 5 1 | 4 4 | . . | 1 . | | 1 1 |”, and under total, for “67” read “61”.

Page 245, table D, reference number 1133, under headings 1 to ,2 4 inclusive, for “| 17 12 | 11 10 | 16 13 | 31 31 | 3 . | 3 3 | 3 3 | . | 5 4|”, read “| 7 12 | 10 10 | 16 13 | 21 31 | 2 . | 5 . | 5 1 | . | 3 6|”, and under total for “337” read “314”.

CORRIGENDA (Continued)

Page 246, table D, reference number 1233, under heading 3, for "3 6" read "3 10"; reference number 1243, under heading 1, 2, 3, for ". ." read ". 1"; reference number 1247, under heading 1,4, for ". 4" read "1 4"; reference number 1259, under total, for "72" read "82".

Page 288, footnote, insert between first and second line, "...dominant character dichætic D. Flies showing the vortex characters are..."

Page 425, table 2, last line, under chromosome number, for "o" read "10".

Page 458, for "EWART" read "EWERT".

Page 490, line 1, for "factor" read "feature".

Page 490, line 19, exponent of e , for " $-\frac{x^2 - 2\rho xy + y^2}{2\sigma^2(1-\rho^2)}s^2$ " read " $-\frac{x^2 - 2\rho xy + y^2}{2\sigma^2(1-\rho^2)}$ ".

Page 492, line 6 below table 1, for " $\log, \frac{1+r}{1-r}$ " read " $\log_e \frac{1+r}{1-r}$ ".

Page 496, line 2 below table 3, for "phase" read "factor".

Page 497, line 8, for ".8300" read ".8333".

Page 497, line 15, for " $t = \frac{c_1}{4}(3 + c_2 A)$ " read " $t = \frac{c_1}{4}(2 + c_2 + c_2 A)$ ".

Page 497, line 22, for " $t = 3f - \frac{3}{2} \frac{p}{1+\mu} - \frac{5p^2\mu}{(1+\mu)^2}$ " read

$$"t = 2f - \frac{1}{2} \frac{p}{1+\mu} - \frac{3p^2\mu}{(1+\mu)^2}."$$

Page 497, line 23, for ".818" read ".757".

Page 497, line 26, for " $t = 3f - 3/2 p - 1/2 \mu$ " read " $t = 2f - \frac{1}{2} p - \frac{1}{2} \mu$ ".

Page 497, line 29, for ".825" read ".744".

Page 497, line 30, insert "not," before the word "extremely."

Page 499, citation of HERTWIG, for "derzeitigen" read "derzeitigen".

Page 512, line 13, for "sygotic", read "zygotic".

Page 535, line 3 from bottom, for "*Fagopyrum*" read "*Fagopyrum*".

Page 560, line 1, for "LEHMAN," read "LEHMANN".

Page 588, heading of table 1, for "508" read "538".

Page 599, line 30, for "BAILY" read "BAILEY".

Page 601, table 1, line 2 under "Mating", for " $ee \text{ } \varnothing \times Ee^b \text{ } \sigma$ " read " $Ee^b \text{ } \varnothing \times ee \text{ } \sigma$ ".

FRONTISPIECE—HUGO DE VRIES

HUGO DE VRIES was born at Haarlem, Holland, February 16, 1848. He attended the Latin school in his native city and in 's Gravenhage until 1866, when he entered the UNIVERSITY OF LEIDEN. From this University he received his doctorate October 6, 1870, defending a thesis entitled "The influence of heat on life phenomena in plants." After finishing his course at Leiden he entered successively the UNIVERSITY OF HEIDELBERG and the UNIVERSITY OF WÜRZBURG in order to continue his studies under the great Masters, HOFMEISTER and SACHS.

From 1871 to 1875 he was Instructor in Natural History at the HOOGERE BURGERSCHOOL, and at the HANDELSCHOOL of Amsterdam. He then accepted an appointment from the DEPARTMENT OF AGRICULTURE of Germany to prepare monographs of some of the larger agricultural crops. Three of these were published, dealing with red clover, beets, and potatoes. This work was done at Würzburg and Halle. In the latter place he became Privat-Docent in the Department of Botany, whose Head was the celebrated plant pathologist, JULIUS KÜHN. It was here and at Würzburg that he developed the plasmolytic method for the study of plant growth, by which it is possible to measure the amount of increase in size due to turgidity of the cells, as distinct from the permanent elongation of the cell walls. In 1877 he returned to Amsterdam as Lecturer in Plant Physiology, and was appointed Professor Extraordinarius the following year and Ordinarius in 1880. On September 16, 1918, he retired after a service of exactly forty years, and has constructed for himself at Lunteren, Holland, a small greenhouse, and has developed an experimental garden in which he is continuing his genetical studies with the evening primroses.

During the early years of his work at the UNIVERSITY OF AMSTERDAM he devoted himself to studies of osmotic pressure in plants, and discovered the isotonic coefficients of salt and other solutions, on which, shortly afterward, VAN'T HOFF based his well known law that dilute solutions are subject to the same laws as gases. These coefficients made it possible to determine osmotic pressure in living cells, expressed in atmospheres, and to estimate the part each constituent in the cell sap takes in producing this pressure.

Besides these fundamental physiological investigations he began almost immediately the studies on heredity, which have served to bring this subject, which was then of wholly comparative and speculative nature, into the domain of experimental investigation.

In 1899 he published a criticism of DARWIN's provisional hypothesis of pangenesis, in a book entitled "Intracellular pangenesis," which has been translated into English by C. STUART GAGER. He developed at the UNIVERSITY OF AMSTERDAM an experimental garden which was one of the first of its kind, if not the first, in existence, and in this garden he developed races of various plants characterized by fasciations, torsions, pitched leaves, and other anomalies, whose

heredity had not been previously studied. Here also he conducted the extensive *Oenothera* cultures whose behavior led to the formulation of the Mutation Theory. The foundation of these cultures was laid in 1879 by the discovery of *Oenothera Lamarckiana* and two of its derivatives growing in a field at Hilversum, Holland. From that time until now he has continued his studies of this curious plant with great zeal, and in recent years has been joined in the work by a large number of investigators in different countries.

In 1901 and 1903 appeared the two volumes of "Die Mutationstheorie," which along with the re-discovery in 1900 of MENDEL's principles of heredity, mark the beginning of a new era in biological science.

In 1904 he accepted an invitation from the UNIVERSITY OF CALIFORNIA to deliver a course of lectures in its Summer School. These lectures were published soon after, under the title "Species and varieties; their origin by mutation." His first address on arriving in America was delivered at the opening exercises of the CARNEGIE INSTITUTION'S STATION FOR EXPERIMENTAL EVOLUTION, on June 11, 1904. In 1906 he again participated in the work of the Summer Session of the UNIVERSITY OF CALIFORNIA, and published his lectures in a small volume entitled "Plant breeding." He also lectured in many other American Universities. His third and latest visit to America took place in 1912, when he delivered one of the invitation addresses at the opening of the RICE INSTITUTE, Houston, Texas. On this visit, accompanied by H. H. BARTLETT, he explored a portion of Alabama in search of large-flowered *Oenotheras*, and also visited COLUMBIA UNIVERSITY, where he was much impressed with the experiments of T. H. MORGAN and the cytological studies of E. B. WILSON. Since that time he has given much attention to applying the principles discovered by MORGAN and WILSON to his genetical results in the *Oenotheras*, especially with respect to the occurrence of lethal factors.

On February 16, 1918, he celebrated his 70th birthday, both at Amsterdam and at Lunteren, and was honored by an arrangement to republish in seven large volumes all his contributions to Dutch, German, American and other scientific journals. This work is entitled "*Opera e periodicis collata*." On June 13, 1918, he delivered his last lecture as Professor of Botany in the UNIVERSITY OF AMSTERDAM, on the subject "From amoeba to man."

It has seemed peculiarly fitting therefore to present this portrait of HUGO DE VRIES to readers of GENETICS at this time. The photograph reproduced here was taken by ELLIOTT & FRY, Ltd., London, on the occasion of the DARWIN CENTENNIAL CELEBRATION in 1909. It is copyrighted by the Photographers, from whom the right to publish here has been purchased.

The reproduction of this portrait of Professor DE VRIES is made possible by a gift from Dr. LIBERTY HYDE BAILEY, who was for many years Director of the NEW YORK STATE COLLEGE OF AGRICULTURE at CORNELL UNIVERSITY and who is Author and Editor of many important works on horticulture and agriculture including such works of special interest to geneticists as "Plant breeding," "Survival of the unlike," "Evolution of our native fruits," etc. In the title of the first-mentioned of these works (first published in 1895) it is believed that the expression "plant breeding" was first used.

THE HEREDITY OF QUANTITATIVE CHARACTERS IN WHEAT

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DATE OF FIRST HEAD

Macaroni \times bread wheat crosses. Algerian macaroni (No. 1) \times Sonora (No. 35)

Bread wheat crosses. Red Algerian bread (No. 3) \times early Baart (No. 34)

Summary; date of first head

HEIGHT

Macaroni—bread wheat crosses. Algerian macaroni (No. 1) \times Sonora (No. 35)

Algerian macaroni (No. 1) \times Algerian red bread (No. 3)

Height in bread wheat crosses, 3 \times 35

Red Algerian bread (No. 3) \times early Baart (No. 34)

Summary; height

WIDTH OF LEAF

Macaroni (No. 1) \times Sonora (No. 35)

Algerian macaroni (No. 1) \times Algerian red bread (No. 3)

Inheritance of leaf width in bread wheat crosses, Sonora (No. 35) \times red Algerian bread wheat (No. 3)

Algerian red bread (No. 3) \times early Baart (No. 34)

Summary; width of leaf

GENERAL SUMMARY

LITERATURE CITED

[Received May 15, 1918]

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INTRODUCTION

This paper forms a report on certain phases of a series of investigations in wheat breeding under the supervision of the writer, in the Department of Plant Breeding of the ARIZONA AGRICULTURAL EXPERIMENT STATION. The work was initiated by the making of a number of

heredity. Local crosses between an Algerian white macaroni wheat, an Algerian red bread wheat and two local white bread wheats, Early Baart a Sonora. The original hybridizations were made at Yuma, Arizona, the spring of 1913, the F_1 was grown at Tucson in 1913-'14 and the F_2 and F_3 on the experimental farm at Yuma in 1915, 1916, respectively. The data concerning time relations, width of leaf, height, rust resistance etc., were, of course, taken in the field. At the time of ripening heads of each plant were harvested and placed together in a paper bag, care being taken to label each bag so that it could be completely identified. All other data were taken in the laboratory of the Department of Plant Breeding at the UNIVERSITY OF ARIZONA at Tucson. The publication and analysis of this data begun some months earlier, has continued throughout the present year by the writer while on leave from the UNIVERSITY OF ARIZONA. The writer herewith expresses his appreciation to the officers and management of the BUSSEY Foundation for laboratory and library facilities throughout the year. He wishes to recall with appreciation the assistance rendered by Dr. E. M. EAST for many valuable criticisms and suggestions, by Mr. DONALD F. JONES who made the original crosses, by Mr. LEONHARDT SWINGLE to whose careful and accurate work may be credited a large proportion of the field and laboratory notes of the second generation, and finally, by Mr. W. E. BRYAN in his efficient assistance with the field and laboratory notes for the third generation.

Since the re-discovery and publication of MENDEL's original papers, the question of paramount interest among geneticists and plant and animal breeders has been that as to whether or not the principles involved in the discoveries of MENDEL are of limited or universal application. Practically all real progress in the study of heredity has arisen through experiments and observations designed to test the validity and universality of MENDEL's laws.

At the present time, the inheritance of a large number of characters, including those both of a qualitative and quantitative nature, in a wide series of both plants and animals, are almost universally considered to be best explained by the Mendelian hypothesis. These include all characters which in the F_2 and subsequent generations, show definite, discontinuous segregation. Most of the cases of peculiar and unusual ratios have been satisfactorily explained as due to multiple factors, lethal factors, gametic coupling, gametic selection, partial sterility, etc.

There are cases, however, which admit of explanation by hypotheses other than those based upon Mendelian principles. Examples may be

cited among characters which may be expressed quantitatively. In many such cases the F_1 is more or less intermediate between the parents, and the F_2 and subsequent generations show segregation, but such segregation as does occur is perfectly continuous. Where a sufficiently large number of variants are grown, there is found every degree of size from the lowest to the highest extreme of the hybrid distribution. The extremes of this distribution may or may not reach or extend beyond the extremes of the parental races.

There are some geneticists who believe that such a type of inheritance is not Mendelian. They advocate the application of the Mendelian principles in many cases, but maintain that we have no proof that Mendelism is universal and that cases such as those described above may be just as easily explained by assumptions other than those of gametic purity and unchanged segregation.

The literature on the subject of the inheritance of quantitative characters has been collected by SHULL (1914) and MACDOWELL (1914), and has been summarized with excellent clearness by these writers. It is therefore not necessary to re-summarize these earlier papers. The results of original research bearing upon the inheritance of quantitative characters which have appeared since SHULL's and MACDOWELL's summaries may now be reviewed briefly.

NILSSON-EHLE (1914) shows a genetic linkage between a factor for yellow glume color and an inhibitor which shortens beard length in oats.

PHILLIPS (1914) crossed Rouen and Mallard ducks which differ greatly in size. The F_1 was intermediate in size between the parents and not more variable than the most variable parent. The F_2 , while still intermediate in average size, was markedly more variable than either the F_1 or the parents.

PUNNETT and BAILEY (1914) in crosses of bantam with larger breeds of fowl found the F_1 intermediate and the F_2 highly variable, transgressing the extremes of both parents. Small F_2 fowl bred together gave an F_3 all of small size; large F_2 individuals bred *inter se* produced altogether large offspring. The F_3 obtained by mating intermediate F_2 individuals was highly variable. They interpret the results as being due to the segregation of Mendelian unit factors and give a factorial scheme to account for the phenomena observed.

HAYES and EAST (1915) crossed flour corn with a flint variety and found that the endosperm character was determined by the mother only, although it was proved that endosperm character, first visible in the next generation could be inherited through the pollen. The authors conclude

that this behavior is due to the fact that the endosperm is produced from a union between two female polar nuclei and one male cell and that the presence of two factors dominates one in either the direction of starchy or flinty endosperm. In other flint-starchy crosses, the ratios were not so definite, due possibly to the difficulty of classifying the seed. It was thought, however, that the same principles were involved as in the previous crosses. Crosses involving grains of different shape were made between rice pop corn, pearl pop corn and a dent corn. The results of these experiments indicated that several factors were involved which segregated in a Mendelian fashion in the F_2 and F_3 . Parental types when once recovered bred true.

EAST (1916 a) records the crossing of *Nicotiana Langsdorffii* and *N. alata* which differ markedly in corolla length. The F_1 was intermediate and no more variable than the more variable parent. The F_2 also had an intermediate average but the variability was much higher than in the F_1 . There was a wide range in the variability of the different F_3 races but they were all lower than in F_2 . He showed by F_3 pedigrees that segregation had occurred in F_2 but did not attempt to determine the number of factors.

EAST (1916 b) in a second paper reports the results of crossing a variety of *Nicotiana longiflora* having the corolla about 93 mm long with another variety of the same species having a corolla length of about 40 mm. He carried the study through the first, second, third, and in a few races as far as the fourth generation, with sufficient numbers to calculate the coefficients of variation in the separate races. The author lays down eight conditions which he assumes the data must fulfill in order to be interpreted as complying with the conditions of Mendelian inheritance. Tables and distributions with the calculated constants are given in detail and the conclusions are that no single phenomenon has occurred which cannot be interpreted as Mendelian.

PHILLIPS (1915) after a study of the results of color inheritance in various duck crosses and pheasant crosses says that "it is almost certain that the ordinary subspecies of the ornithologist is very far from being a unit variation."

Since the work of JOHANNSEN on the effect of selection in beans, there has been no similar work with plants which can compare in volume and significance with that of FRUWIRTH (1915). FRUWIRTH followed the system of pure line selection as practiced by JOHANNSEN. Choosing a variety of *Lens esculenta* with flecked seed, he endeavored through selection to bring about greater flecking on the one hand and the diminu-

tion of the flecks on the other. After 13 generations he had made no progress in either direction. Chevrier beans (*Phaseolus vulgaris*) produce seeds which, for the most part, have seed coats of a slightly greenish color rather than creamy white but a few seeds are white on one or both sides. It was attempted, through selection within a pure line, to secure complete inheritance of the green type. Though carried out for 14 generations no change was produced. In a race of vetch which produced both green and cream-colored seeds on the same plant, he tried for 10 generations to fix the green coloration by selection but made no progress. Likewise two years selection of yellow seed made no progress in the direction of fixing the type. In a Victoria pea variety with yellowish green and yellow seed three years of selection was without effect. In a variety of Soja bean having lighter and darker brown seed, three years of selection could make no progress in either direction of darker or lighter seed coats. In a certain variety of *Pisum arvense* the seeds are variable in color. They may be pure yellowish green, or yellowish green with violet flecks or bands, or the violet color may be so extended as to leave the yellowish green appearing only as flecks, or finally the violet color may prevail altogether. FRUWIRTH endeavored by selection to increase the amount of violet color in the seeds on the one hand and to reduce it on the other. In the selection for more violet color in the seed coats, 10 generations produced no results. The results of the selection in the opposite direction can best be given in FRUWIRTH's own words as follows (FRUWIRTH 1915, p. 200):

"In beiden JOHANNSEN'SCHEN Linien I und A ist die Anlage zur Ausbildung violette Farbe der Samenschale vorhanden, die Anlage ist aber stark modifikabel und ausserdem sind beide Linien geneigt spontan Zweige abzuspalten, in welchen diese Anlage ihre Wirksamkeit ganz (in I die Zweige II von Ernte 1909, und IV von 1910 Ernte) oder fast ganz (in I der Zweig III der von Ernte 1908 abgeht und die Auslese A) eingebüsst hat. Eine Neigung rein violett-samige Zweige abzuspalten, besteht nicht."

"In beiden JOHANNSEN'SCHEN Linien ist die Anlage zur Ausbildung violette Farbe in der Hülsenschale vorhanden, und zwar ist die Anlage—sowie jene violetter Farbe der Samenschale—stark modifikabel. In beiden Linien ist die Neigung vorhanden, spontan Zweige abzuspalten, in welchen die Wirkung der Anlage durchschlagend, ohne Modification auftritt, so dass dann nur violette Hülsen gebildet werden. Violette Färbung der Samenschale ist ganz unabhängig von violetter Färbung der Hülsenschale."

"Auslese nach grüner Farbe der unreifen Hülse ist wirkungslos, Auslese nach violetter Farbe derselben nur dann—und dann sofort—von einer

Wirkung begleitet, wenn spontan ein violetthülsiger Zweig abgespaltet worden ist."

In a selection carried out upon a variety of lupine (*Lens esculenta*) having mottled seed, FRUWIRTH sought by selection to produce both dark- and light-seeded strains. Six years selection in one direction and eight years in the other produced some divergence in the selected lines but was not effective in producing either self-colored dark- or light-seeded races.

In a variety of vetch which normally produced either greenish or cream-colored seed (see selection experiment described above) after five generations of self-fertilization, there appeared in the harvest of 1910, 2 plants having mottled seeds. In 1912 after 7 generations of self-fertilization and selection the same line produced 4 plants having mottled seeds. Finally, "trat diese Variation auch als Variation einer ganzen Pflanze bei 5 Individuen der Ernte 1910 auf, nach 9 Generationen aus Selbstbefruchtung, fünf in der Linie, vier während der vorangegangenen Massenauslese." All mottled seed bred true.

In selection work with Soja beans one or two spontaneous variations were observed. All effects of selection (from a mass lot), however, were produced in the first year. The spontaneous origin of a white-flowered vetch is also noted.

White mustard (*Sinapis alba*) with which FRUWIRTH worked, produces both yellow and brown seed. After eight years of selection of close-fertilized seed, he was unable to fix the type or even materially to diverge the tendency in one direction or the other.

In extensive selection experiments with oats which for some characters were carried through ten generations he decides that selection within pure lines is without effect.

FRUWIRTH (1915, p. 450) finally sums up by saying:

"Bei einer Reihe von äusseren Eigenschaften zeigte sich durchweg, dass in einer JOHANNSEN'SCHEN Linie bestimmt gerichtete Auslese auch bei Fortsetzung durch eine grössere Zahl von Generationen keine Änderung des Liniencharakters mit sich bringt."

MACDOWELL (1915) has reported the results of selection experiments upon a race of *Drosophila* which possessed more than the normal 4 bristles on the thorax. The average number of bristles increased for 6 generations of selection. The same selection was carried on for 5 more generations without additional effect. The author concluded that there were several accessory factors limiting extra bristles which were gradually eliminated by selection. MACDOWELL has also shown a very

strong correlation of extra bristles with body size. The present writer strongly suspects that the real factors here concerned were size factors and that MACDOWELL's extra bristle selection was merely an indirect means of selecting for larger size.

The paper by YUZO HOSHINO (1915) on the flowering time of peas and rice has been the subject of much interesting recent comment. HOSHINO crossed early- and late-blooming varieties of peas. He found that the variation behaviors of the F_1 , F_2 , F_3 and F_4 races (detailed distributions of which are given) could for the most part be interpreted by assuming the Mendelian segregation of two allelomorphous pairs, A and a , which determined early- and late-blooming respectively and two modifiers B and b . Those variation behaviors which could not be explained by these factors, he supposed to have been caused by a "contamination" of genes. What he means by contamination of genes is not clear for he distinctly states that he does not refer to such a contamination as is assumed by CASTLE in rodent crosses. He suggests "secondary factors." This is the same as assuming additional factors of secondary importance such as are assumed by NILSSON-EHLE in the report of his *compactum*-squarehead-Landweizen wheat crosses.

HOSHINO has also shown a gametic coupling of early-blooming with white flowers and late-blooming with red flowers. This coupling is broken (by physiological interference or crossing over) approximately 1 time in 7.

In crossing early- with late-shooting rice varieties he finds the F_1 intermediate, the F_2 showing strong segregation. The behavior of the F_3 and F_4 races were such as would be normally expected of segregating Mendelian factors.

CASTLE (1917) has re-stated certain data and conclusions previously published (CASTLE 1912, pp. 163-168). In crossing + variants of hooded rats with wild rats he found that "wild" was dominant in F_2 and that the hooded extractives of the F_2 were often higher in hood grade than were their hooded grandparents. In crossing "mutant" hooded rats (a race which suddenly appeared with a very high + hooded condition) with wild rats, the F_1 was of the wild type but the hooded extractives of the F_2 did not drop lower than the range of the original "mutant" race. CASTLE concludes that these facts cannot be interpreted as Mendelian and must be explained as the results of changes in a single unit factor.

The present paper is offered as the first in a series of further contributions to the knowledge of the inheritance of quantitative characters. Wheat has proved an especially favorable subject for such an experi-

ment inasmuch as its small size renders feasible the production of large numbers without prohibitive expense and the fact that it is close-pollinated greatly simplifies the genetic analysis of the F_2 and subsequent generations.

The characters here studied are the date of the appearance of the first head on each plant, the total height of the plants measured in centimeters from the ground to the top of the tallest head (not including beards) and the width of the broadest leaf.

MATERIAL AND METHODS

A brief description of the four varieties of wheat used may be given as follows:

Algerian macaroni (No. 1)

Late, tall; stems large, stiff; leaves broad, dark green, medium width; heads large, cylindrical, flattened, long; glumes bearded, pubescent, light straw yellow; grain large, mostly translucent light amber, and very hard, but with some grains having spots of opaque starch in the endosperm. Originally obtained from R. MARIE, Algiers, Algeria.

Algerian red bread (No. 3)

Late, tall; stem medium in size; leaves medium in width and color; heads medium size, square; glumes bearded, smooth, light straw yellow; grain red, medium soft, opaque. Originally obtained from R. MARIE, Algiers, Algeria.

Early Baart (No. 34)

Early, low; stem medium in size; leaves medium width, medium green; heads medium size, square; glumes bearded, smooth, light straw yellow; grain white, medium soft, medium size, opaque. Originally obtained locally.

Sonora (No. 35)

Early, low; stem medium in size; leaves broad, light green; heads cylindrical, square, medium size; glumes beardless, pubescent, reddish brown; grain white, opaque. Soft. Originally obtained locally.

All planting was done with a nursery row machine by which each grain was covered 2 inches deep and spaced 3 inches in rows 10 inches apart. There were fifty hills in each row. Strips of barley were planted on either side of the plot in order that the end plants should not have

more space than those within the plots. All plants of the pure varieties grown in 1914 were from mother plants which were selected from the 1913 general mass cultures as true to the types of their respective varieties. Of these selected 1913 plants there were 14 of macaroni (No. 1), 3 Algerian red bread wheat (No. 3), and 5 early Baart. The head records for Sonora (No. 35) in 1914 came from 12 typical heads of this variety selected from a mass culture. In 1915, of the 9 nursery rows of pure macaroni (No. 1), 6 were plant rows from the previous year's culture and 3 were from a mixture of seeds resulting from threshing together a number of typical heads of this variety selected from a field culture. The 3 nursery rows of No. 35, 1 of No. 3 and 1 of No. 34 were plant rows from the previous year's harvest. In 1916, 5 of the nursery rows of No. 1 came from a single mother plant in 1915 (No. 52-4-1-4) and the remaining 2 from a single other 1915 mother plant (No. 3-12-1-5). The 5 nursery rows of each of the other varieties originated from single plants in 1915 as follows: No. 35 from No. 35-11-1-4; No. 3 from No. 32-2-38; No. 34 from No. 1-13-3-1-24. In all of the discussions, the word culture is used in the sense of a group of plants, grown in a single nursery row and originating from a single mother plant of the previous season. This applies alike to the pure varieties and hybrids. The exception in the case of the 3 nursery rows of mass-selected macaroni, grown in 1915, has been noted. The expression "pure race" is often used to distinguish plants belonging to one of the parental varieties from those of hybrid origin.

The statistical methods used in these investigations were those commonly employed by biometricians. The constants used were the arithmetical mean, standard deviation and coefficient of variation. The means were calculated to the nearest unit employed in the taking of the original data. The standard deviations were calculated from the mean class as a mean, i.e., with the middle of the mean class as the assumed mean, no correction being made for the true mean. This was considered sufficiently accurate in view of the fact that different plant rows of the same pure race (pure line originating from a single mother plant) often showed more difference in standard deviation in the same season than could possibly arise from failure to correct for the true mean. An example will suffice. All of the plantings of pure No. 3 (Algerian red bread) arose from the seeds of a single plant in 1914. In 1916 there were 5 plant rows of this culture grown in different parts of the experimental plots for comparison with the various hybrids into which this culture entered. The data for height and the statistical con-

stants calculated therefrom by various methods are given below. The original measurements were made to the nearest centimeter and in the summation of the data the classes were made to include 5 cm with the middle points at 2.5 and 7.5, thus 62.5, 67.5, etc.

TABLE 1 A
Height of pure No. 3, 1916, in centimeters.

Row No.	45 to 49	50 to 54	55 to 59	60 to 64	65 to 69	70 to 74	75 to 79	80 to 84	85 to 89	90 to 94	95 to 99	100 to 104	105 to 109	110 to 114	115 to 119	120 to 124	125 to 129	130 to 134	135 to 139	140 to 144	145 to 149	150 to 154
105A ...	1*												1	3	3	19	8	8	2			
105B ...															2	2	4	10	13	15	3	
105C ...															1		2	12	21	10	3	1
105D ...															1	8	18	14	6	1		
105E ...																	7	13	9	10	6	5

* Not used in calculation of constants given in table 1 B.

TABLE 1 B
Statistical constants.

Row No.	Number of variants	True mean (A)	Mean used in the calculation of σ used in the discussions (B)	Approximate mean given in the tables and discussions	Standard deviation calculated on (A)	Standard deviation calculated on (B)
105A	44	122.85	122.5	123	6.4	6.4
105B	49	135.00	137.5	138	7.0	7.3
105C	50	137.40	137.5	138	5.8	5.8
105D	48	129.50	127.5	128	5.2	5.5
105E	50	138.50	137.5	138	7.7	7.7
Averages and totals	243	132.65	132.5	133	8.5	8.6

Now the greatest difference in standard deviation arising from different methods of calculating was .3 or about 3.5 percent of the average standard deviation, whereas the greatest difference between the different lines was 2.5 (that between 105D and 105E) or 29.4 percent, a little over eight times the error introduced by the different methods of calculation. In view of such facts it was not considered worth while to waste time in accuracy of calculation which could not possibly add any significant value to the constants so obtained.

Although the probable errors of a large proportion of the constants here given have been calculated they are not given in the text on account of lack of space and the difficulty of placing them in compli-

cated tables of distribution, etc. In nearly every case, however, in which the reader is interested, the probable errors can readily be calculated from the data given. In the F_2 hybrids most of the cultures had from 85 to 95 individuals and in the F_3 , from 40 to 48.

It has been necessary to devise some means of comparing the variability of a series of hybrid races with their pure line parents, each of which may perhaps be grown in several different plant rows in different parts of the experimental plots. Moreover, if we accept high variability as a measure or indication of heterozygosity, it will be of interest to compare the variability of second generation hybrids with the third generation (F_3). In close-pollinated plants like wheat, as the average of heterozygosity certainly decreases from generation to generation, the average variability of plant populations (populations arising from single mother plants) should also decrease. This average increase in homozygosity with respect to any one character is, however, not uniform in all lines. The recombinations may be such that an F_2 plant is just as heterozygous with respect to the factors governing height, for instance, as was its F_1 parent and the same may be said of certain individuals in the comparison of the F_3 plants with their F_2 parents. We will therefore have some F_2 plants just as heterozygous as their F_1 parents that will give rise to cultures of F_3 which are just as variable as were the F_2 cultures, but the majority of the F_2 plants will be less heterozygous than their F_1 parents and will therefore give rise to F_3 cultures less variable than were the F_2 cultures. Now since the quantitative characters concerned, as well as the variability of the same, are subject to environic modification (see behavior of pure lines in table 1) there must be some means of comparing statistically the variability of the F_3 cultures with the F_2 cultures in order to demonstrate this general decrease of variability in the succeeding hybrid generations.

Three methods are available as follows:

- (a) Throw all the cultures of a given generation into a single population and calculate the standard deviation of the same.
- (b) Superimpose the means of the several hybrid cultures, sum the equal deviations on each side of this mean and calculate therefrom a standard deviation for the whole series.
- (c) Calculate the standard deviation and coefficient of variation of each hybrid culture separately and show the average and distribution of these constants.

These methods and the value of the constants so obtained will now be discussed in order:

(a) The standard deviation calculated by this method from a population consisting of several plant rows of a single pure line is always greater than the average of their standard deviations taken separately. This is caused not necessarily by differences in the standard deviations of the plant rows entering into the total population (these may be all identical) but by differences (enviromic) in the means of the several rows whereby the distribution of the population as a whole is much broadened. The distribution of this total population and the standard deviation derived from it are therefore measures of the total effects of the given different environments in modifying the character concerned. If now we are dealing with an F_2 generation all of which originated from genetically equivalent F_1 plants, part of the differences in the F_2 plants would be due to enviromic effects and part to the effects of genetic recombination. The distribution and standard deviation of a hybrid population calculated by method (a) would therefore give the total combined effect of environment and recombination in producing variability. When now we come to consider an F_3 population arising from genetically unequal F_2 plants we simply re-measure (if we plant all the seeds of all of the F_2 plants or a sufficiently large random sample) the influence of the same factors as were measured in the F_2 , i.e., the sum of the effects of environment and all of the factors entering the cross from the original parents. We cover up the possibility of discovering any decrease in the heterozygosity of the F_2 plants since differences in the means of the F_3 cultures, due to the genetically different parents, will have the same effect in broadening the distribution of the total population, as differences in the individuals of a single highly variable culture.

(b) The method of superimposing the means introduces a small but unavoidable mathematical error where the standard deviation is used as a measure of the average variability of a number of separate cultures. It is well known, however, that where the means differ, the standard deviation is not a good measure of comparative variability. In order to overcome this difficulty and obtain abstract numbers which may be compared, the coefficient of variation has been devised. This is the percentage which the standard deviation is of the mean. It is therefore apparent that a given deviation from the mean has more weight in the determination of the coefficient of variation when it is a deviation from a small mean than when it is a deviation from a large mean. When now we superimpose small means and large means we give equal values to deviations which are of unequal value in determining the coefficient of variation. Hence if our data have to do with cultures differing widely

in their means, where the coefficients of variability rather than the standard deviation must be used in the comparison of variabilities, we are not justified mathematically either in averaging standard deviations or superimposing means. As a matter of fact, however, it may be said that the error introduced by this means is not large. Taken alone, however, the method of superimposing the means has one serious fault. It covers up wide differences in the variability of different individual F_3 cultures. For the purposes of genetic analysis it is necessary to know whether all of the F_3 cultures have decreased in variability or whether this decrease is confined to the offspring of certain only of the F_2 plants. It is therefore necessary to calculate the standard deviations and coefficients of variation of each of the cultures separately.

(c) Since, as just stated, a knowledge of the distribution of the coefficients of variation of a series of hybrid cultures is probably even more important than a single general expression of the average variability as a whole, method (c) which gives all of these details is usually to be preferred.

In general the coefficient of variation was used as a measure of variability. In time relations, however, this is difficult on account of the necessity of selecting arbitrarily some point from which to estimate the means. In the case of the date of first heading, if some date in March, say the first or fifteenth were chosen, it was feared that the differences in means would be so great as to unduly distort the coefficients of variation. One may readily see that the later such a basal date be chosen the greater will be the distortion on this account. On the other hand, if the chosen date be moved backward, the various means, in comparison with each other, approach unity, and the coefficient of variation becomes then more and more dependent upon the size of the standard deviation. Although all of the plots were planted within a period of seven days in the fall and all came up at approximately the same time, it would be questionable whether the total vegetative period would be the best basis of a determination of the variability of date of first heading on account of the fact that some strains were more active in winter than others and were therefore given unequal starts in the rapid vegetative period of spring. In view of these difficulties it was decided to use the standard deviation (expressed in days) alone as the measure of variability in all time relations.

In the studies on size relations, the coefficients of variation only are given.

Where averages of a series of standard deviations are given, or

standard deviations are calculated from artificial populations produced by superimposing the means of different races, such fact has been expressly stated, but it must not be understood that the writer would infer that these are strictly comparable mathematically to an average of a series of coefficients of variability, for reasons already given. Rather than true arithmetical averages, such means should be considered as foci around which the distribution of the given series of constants (here standard deviations) cluster, and therefore form, as it were, a locus for thinking specifically.

DATE OF FIRST HEAD

The dates of the first head of the parents and the F_1 plants in 1914 were not taken.

Macaroni \times *bread wheat* crosses. *Algerian macaroni* (No. 1)
 \times *Sonora* (No. 35)

In 1915, 3 pure races of No. 35, 9 pure races of No. 1, and 37 cultures of $(1 \times 35) F_2$, were grown at Yuma. The following results were obtained:

TABLE 2 A
Date of first head in F_2 of cross 1×35 and in the parent strains, 1915.

	Number of cultures	Number of individuals	Average dates of first head	σ of population	Average σ of cultures
Pure No. 35...	3	168	March 17	2.14	1.66
$(1 \times 35) F_2$...	37	2546	" 27	4.00	3.56
Pure No. 1...	9	650	" 31	3.30	1.87

TABLE 2 B
Distribution of standard deviation of cultures.

	.75	1.25	1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75
Pure No. 35.....		1	1	1							
$(1 \times 35) F_2$				2	7	12	5	8	2	1	
Pure No. 1.....		1	5	2		1					

The 37 hybrid cultures were from the seed of the 37 F_1 plants secured in 1914 which were sown in plant rows in 1915. It should here be noted that the standard deviation of the whole population is markedly higher than the average standard deviation of the plant rows taken separately. This was also true of the pure races and can be attributed in part to the place variation of the different plant rows. Part of this difference may

also be due to slight differences in the genetic composition of the individuals of the parental varieties used in the original cross. However, these individuals, although not all belonging to one pure line, in their respective varieties, were carefully selected as belonging to the type of the variety which they were to represent. The differences between the average standard deviation of the pure lines taken separately and of their respective populations is therefore an approximation of the error introduced by place variation (modification) and whatever genetic differences there might have been in the several individuals of the parental cultures.

The greater variability of the hybrid cultures as compared with the parental varieties is in accordance with what would be expected from the recombination of genetic factors in the F_2 generation. The mean of the hybrid cultures was 3 days later than the mean of the parents and 4 days earlier than the late parent. The heading dates of both parents and of the F_2 cultures may be summarized as follows:

TABLE 3
Date of first head in (1×35) F_2 , 1915.

Cultures	March										April									
	15	17	19	21	23	25	27	29	31		2	4	6	8	10	12	14	16	18	20
Pure No. 35.....	16	18	20	22	24	26	28	30	1		3	5	7	9	11	13	15	17	19	21
(1×35) F_2	25	85	47	7	4						98	86	42	17	8	1	2			1
Pure No. 1.....	4	18	74	21	403	796	306	403	266		134	81	54	5	2					
						11	78	153	132											

Means of cultures.

Pure No. 35.....			2	1																
(1×35) F_2					1	7	17	12												
Pure No. 1.....							1	4	1	2	1									

From the 2546 F_2 plants, 230 were selected and planted in plant rows at Yuma in the fall of 1915. These selections were, for the most part, based upon economic characters. However, the dates of first heading of the plants in the spring of 1915 varied from March 15 to April 9 and thus furnished material for the study of the segregation of the factors relating to time of heading.

For comparison of the parental varieties with these F_3 hybrids, 7 pure cultures of No. 1 and 5 pure cultures of No. 35 from plants selected as types from these same varieties of the previous year, were grown. The results may first be summarized as follows:

TABLE 4
Date of first head in (1×35) F_3 , 1916.

Culture	Number of cultures or plant rows	Number of individuals	Average date of first head	σ of total population	Average σ of culture
Pure No. 35...	5	247	March 25	1.34	1.27
(1×35) F_3 ...	230	9772	April 11	6.24	3.14
Pure No. 1....	7	343	April 15	1.99	.91

Distribution of standard deviation.

Culture	.25	.75	1.25	1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75	6.25	6.75	7.25
Pure No. 35.....	1	3	3												
(1×35) F_3		2	9	20	38	35	45	41	17	8	9	4	1		1
Pure No. 1.....		2	1	1	1										

The increase in the variability of the F_3 population of hybrids over the F_2 population is striking and surprising. Knowing that only selected individuals of the F_2 were planted, one, at first thought, might be inclined to attribute this to the selection of extremes from both ends of F_2 as parents, but observation of the column showing number of cultures in table 4 will show that the distribution of F_2 parents forms practically a normal curve. One can therefore only attribute this increase to climatic differences in the two seasons which emphasized the effects of extreme combinations more in 1916 than in 1915, or else to the following, which probably accounts for the greater part of the increase. It will be noted that the standard deviations of both the populations and cultures, averaged separately, of the parental varieties, was less in 1916 than in 1915, and also that the same was true of the average standard deviation of the separate cultures of F_3 as compared with that of the separate cultures of F_2 . These facts indicate that the season of 1916 did not emphasize the extremes either in the pure cultures of that year or in the F_3 cultures taken separately, or at least that in the latter case the increasing homozygosity of the F_3 over the F_2 was a little more than able to offset this effect and thereby reduce the variability of the F_3 cultures as compared with the F_2 cultures taken separately. Now in this increase in homozygosity of the F_3 cultures probably lies the increase in variability of the population as a whole. We have already seen that the heterozygotes here tend to take an intermediate position. Hence as the percentage of heterozygous forms decreases with the approach toward homozygosity, the percentage of intermediate types will grow less, i.e., the curve will be flattened, and the standard deviation of the population, thereby slightly increased.

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TABLE 5
Date of first head in (1×35) F_3 , 1916.

	Number of cultures	March										April										May					Number of individuals	
		15	17	19	21	23	25	27	29	31	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	2		4
Pure No. 35.....	5	16	18	20	22	24	26	28	30	1	3	5	7	9	11	13	15	17	19	21	23	25	27	29	1	3	5	7
(1 × 35) F ₃	230			1	3	8	161	69				4																
Pure No. 1.....	7	3	26	46	43		267	136	356	432	767	693	863	2	2893	2156	578	103	355	103	69	48	20	1	3	1		

Average dates of heading of separate plant rows.

Pure No. 35.....	5																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
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A summary of the distribution of the dates of first head in the plants of the parental cultures and the F_3 hybrids is shown in table 5.

It should now be noted that, considering individual plants, there were among the hybrids, 29 plants earlier than the earliest of No. 35 and 293 plants later than the latest of pure No. 1. Moreover, considered as cultures, there were three cultures whose average date of first head was earlier than the earliest average of any of the cultures of pure No. 35 and that there were 19 cultures averaging later than the latest pure culture average of No. 1. There were in fact three cultures whose average date of first head was later than the latest individual of pure No. 1. Does this indicate that by recombination we may be able to isolate races which are earlier than the early parent and later than the late parent?

Table 6 shows the distribution of the F_3 individuals and cultures arranged according to the date of first heading of the parent F_2 plants. \oplus = the date of the first head on the selected F_2 parent. \circ = the average date of the population arising from such parents (reading horizontally). In the same grouping of cultures there are also shown the distribution of the means of the F_3 cultures taken separately and the distribution of the standard deviations of these cultures. The first vertical column at the left shows the number of F_2 plants (hence F_3 cultures) in each category. In a vertical column are also shown the average of the standard deviations of the cultures taken separately in that category.

Table 7 shows the distribution of the F_3 individuals and cultures arranged according to the means of the F_3 cultures. \circ = the average date of first head of the cultures going to make up the population in that group (horizontal). This table also shows the distribution of the selected F_2 plants which were the parents of the several cultures making up the corresponding culture groups. The distribution of the standard deviations of the several races taken separately which make up its corresponding category is given. The vertical columns are the same as in table 6.

Table 6 shows us that the differences observed in the date of first heading of the individual plants of F_2 were largely genetic, since their offspring (F_3) exhibits but little regression toward the general mean. Again the same thing is perhaps better shown in table 7 where the F_3 cultures are grouped and arranged in accordance with their own means. We then have the distribution of the parents of these groups of F_3 cultures. It will be observed that in no case does the distribution of the parents, for any group of F_3 means extend beyond the normal limits of

TABLE 6
Date of first head in $(1 \times 35) F_2$, 1916. Distribution based upon date of first head of the selected F_2 parents.

F ₂ individuals																													
Number of cultures	March								April																				May
	15 16	17 18	19 20	21 22	23 24	25 26	27 28	29 30	31 1	2 3	4 5	6 7	8 9	10 11	12 13	14 15	16 17	18 19	20 21	22 23	24 25	26 27	28 29	30 1	2 3				
1	+		11	9	8	0	13	1		1	6																		
5		+	3	12	25	25	81	25	28	8	10	2							1										
7			+	3	10	9	88	51	54	32	14	11	6		10	1													
41					2	1	81	46	163	225	348	210	226	0	363	93	18	1	4			1	1						
60						1	+	4	9	102	144	235	268	336	1	867	504	88	11	29	9	5	6						
37							+		3	3	12	92	116	78	0	362	233	55	3	39	9	12	6	3		1			
40								+	1	6	10	54	61	85	1	630	580	153	21	95	30	17	8	6	1	2			
28										+	1	8	22	27	333	214	128	40	113	31	34	16	6			1			
6																	0	71	7	38	7	17	2	4	1				
2											+						49	0											
2												+						3	18	13	0					5% = 1			
1													+					1	17	0									

+ = Selected F_2 parents.

O = Mean of F_2 group.

Number of cultures	Average σ of F_2	Means of F_2 cultures																	
		March								April									
		25	27	29	31	2	4	6	8	10	12	14	16	18	20	22	23	24	25
1	5.21	1																	
5	3.88	3																	
7	3.83																		
41	3.39																		
60	3.00																		
37	3.21																		
40	3.00																		
28	2.81																		
6	3.34																		
2	2.78																		
2	3.51																		
1	2.58																		

TABLE 6 (continued)
Date of first head in (1×35) F_3 , 1916. Distribution based upon date of first head of the selected F_2 parents.

Number of cultures	Standard deviation of F_3 cultures													
	.75	1.25	1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75	6.25	6.75	7.25
1					2		1		1	1	1			
5														
7			1			2		3				1		
41			5	3	6	10	10	3		1	2			1
60	1	5	6	8	6	15	10	4	2	3				
37	1	2		7	6	5	10	4	1	1				
40		2	3	10	7	7	5	1	2	2	1			
28			5	9	3	3	5	1	2					
6				1	1	3				1				
2					2									
2					1			1						
1					1									

variation of the most variable parental culture. If the differences in the means of the F_3 cultures in tables 6 and 7 are due to genetic causes, one would expect the intermediate cultures to be more variable than the extremes, thus assuming that the extreme cultures are more nearly homozygous than those which are intermediate.

Now noting the distribution of standard deviations in the F_3 cultures as given in tables 6 and 7 and the average of the standard deviations for separate cultures as shown in the vertical columns, we are unable to discover such a decrease in variability toward the extremes. In the present material, however, this is not surprising for the following reason: No. 1 and No. 35 differ in so many genetic factors that there is an extremely wide range in the products of their recombination. As a matter of fact many of these recombinations are so radical and unbalanced that they are no longer automatic (i.e., are unable to give rise to a living organism). Hence there is a large percentage of sterility in the F_2 and later generations. Now the recombination of factors which govern (by their interaction) the time of heading in this particular cross are likely so many and so widely different that all of the possible recombinations would give a range of heading time far beyond (both toward the early and late extremes) the limit of physiological possibilities of a normal wheat plant. Hence in the range of variation observed in the F_2 or F_3 of this cross we have only a small section taken from some part of the larger theoretical curve. It would therefore appear much flatter than the corresponding curve of a pure race and there would be but little difference in the heterozygosity, hence, variability, i.e., standard

TABLE 7
Date of first head in (1×35) F_2 , 1916. Distribution based upon the means
of the F_2 culture.

F ₂ individuals																														
Number of cultures	March														April														May	
	15 16	17 18	19 20	21 22	23 24	25 26	27 28	29 30	31 1	2 3	4 5	6 7	8 9	10 11	12 13	14 15	16 17	18 19	20 21	22 23	24 25	26 27	28 29	30 1	2 3					
4		3	23	34	31	0	59	6	9	2	9	1							1											
1			1	4	6	0	18	13	6	1																				
4				2	1	0	88	32	32	10	1				1	1														
6			2	4	3	0	62	40	65	45	20	11	9		7															
9				3		0	28	22	101	95	66	17	7		8	3														
13					1	0	15	12	75	117	187	73	43		50	13	1													
18						0		6	28	79	229	150	128		135	29	2	1	2											
21						0		5	10	38	161	159	209		288	64	5		1											
33						0	1	1	23	64	167	269	0	1	618	200	21	1	6	3	1									
56						0		1	8	32	110	166	1	1175	788	137	12	43	8	10	3	3								
39						0			1		10	17			545	768	230	27	78	30	9	6			1					
8						0					1	7	1		28	153	46	33	43	5	9	6	1							
13						0					1	2	1		27	128	112	10	122	44	31	12	6	2	1					
3						0									6	11	29	17	26	5	30	4	6		1					
3						0										4	10	3	43	7	28	15	4							

0 = Mean of group.

		Distribution of F_2 parents													
Number of cultures	Average σ of F_2	March							April						
		15 16	17 18	19 20	21 22	23 24	25 26	27 28	29 30	31 1	2 3	4 5	6 7	8 9	
4	4.84	1	3												
1	3.07			1											
4	2.70		1	2		1									
6	3.77			1	3		2								
9	2.80				1		5	3							
13	3.34					10	3								
18	3.43					7	7	3	1						
21	3.63					7	10	3	1						
33	3.31					5	14	8	6						
56	2.85					3	15	16	14	8					
39	2.59					1	7	6	10	12	2	1			
8	3.47						1		5	1	1				
13	3.56								3	4	3	1		1	
3	4.14								1	1				1	
3	3.24									2				1	

TABLE 7 (continued)

Date of first head in (1×35) F_2 , 1916. Distribution based upon the means of the F_2 culture.

Standard deviation of F_2 cultures.

Number of cultures	.75	1.25	1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75	6.25	6.75	7.25
4							1		1	1	1			
1						1								
4			1	1	1			1						
6					2	2		1					1	
9	1	1	1	2		1	1	1			1			
13			3	1		3	3	1	1	1				
18			1	1	4	4	5	1	1	1				
21					3	8	5	3		1	1			
33			2	7	5	5	9	3	1					1
56	1	6	6	9	8	12	9	3	1	1				
39		2	5	12	10	5	3	1	1					
8				3		2	1			2				
13				1	3	3	1		2	1	1			
3					1			1		1				
3			1				1	1						

deviation, of the cultures arising from individuals selected from either the middle or extremes.

Bread wheat crosses. Red Algerian bread (No. 3) \times early Baart (No. 34)

In 1915, 1 culture of pure No. 3, 1 culture of pure No. 34 and 6 plant rows of the F_2 of 3×34 were grown. These hybrid rows were from the 6 F_1 plants of this cross obtained in 1914. As noted above, dates of first heading were not taken in the F_1 plants. A summary of the results in 1915 is given in table 8:

TABLE 8
Date of first head in (3×34) F_2 , 1915.

	Number of cultures	Number of individuals	Average date of first head	σ of population	Average σ of cultures
Pure No. 3 ...	1	42	March 28	1.60	1.60
(3×34) F_2 ...	6	538	March 23	3.98	2.95
Pure No. 34...	1	93	March 16	1.75	1.75

Distribution of σ of separate cultures.

	.75	1.25	1.75	2.25	2.75	3.25	3.75	4.25	4.75
Pure No. 3.....			1						
(3×34) F_2		1	1	1	1		2	1	
Pure No. 34.....			1						

As previously, it may be noted again that the standard deviation of the hybrids both as a population and as separate cultures was higher than that of the parental varieties. The mean of the F_2 hybrid population was only 1 day later than the mean of the parents. The heading dates of the populations of parental cultures and F_2 hybrids may be given in table 9.

TABLE 9
Date of first head in (3×34) F_2 , 1915.

	March											April		
	9	11	13	15	17	19	21	23	25	27	29	31		
Pure No. 3.....	10	12	14	16	18	20	22	24	26	28	30	1	2	3
(3×34) F_2	1			18	33	61	136	47	62	130	40	8	2	
Pure No. 34.....				53	33	6		1						

Means of cultures.

Pure No. 3.....										1				
(3×34) F_2							2	3		1				
Pure No. 34.....				1										

From these 538 F_2 plants 112 were selected, for economic reasons, for planting in the fall of 1915. For comparison 5 cultures of each of the parental varieties were also grown. These were selected from typical plants of the parental varieties of the previous season. The range of dates of first heading of the selected F_2 plants extended from March 10th to the 29th, thus covering 19 of the 23 days of total variation of the F_2 . The first summary of results are given in table 10.

TABLE 10
Date of first head (3×34) F_2 , 1916.

	Number of cultures or plant rows	Number of individuals	Average date of first head	σ of total population	Average σ of cultures
Pure No. 3....	5	242	April 13	1.52	.82
(3×34) F_2 ..	112	5321	April 5	6.43	2.95
Pure No. 34...	5	244	March 25	3.10	2.17

Distribution of standard deviations.

	.25	.75	1.25	1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75	6.25
Pure No. 3.....	2	2		1									
(3×34) F_2	2	2	7	14	15	15	7	10	14	8	9	4	3
Pure No. 34.....		1			2	1	1						

The general features of this table are the same as those for the other crosses, namely, that the average standard deviations for the cultures are

less than those of their respective populations and that the hybrid cultures are much more variable than the pure lines. Moreover, as in the comparison of tables 1 and 4 we here note also an increase in the variability of the F_3 population of hybrids over that of the F_2 . (Compare tables 8 and 10.) The failure of the average standard deviation of the hybrid cultures to decline from 1915 to 1916 should be noted. Does this indicate a lack of progress toward homozygosity?

Such an inference would be natural were it not for the peculiar behavior of the parental pure race No. 34.

It will be observed that the variability of this race was strongly increased in 1916 over 1915, although all of the 5 cultures belong to one and the same pure line, i.e., the single pure line grown the previous year, which had originated from a single plant in 1914. Perhaps the same factors which caused this increase in the variability of the pure line No. 34 were also able to increase the variability of the hybrid cultures which arose from No. 34 as one parent and that this influence upon the variability was sufficient to offset that of increasing homozygosity and thus maintain the variability for the two seasons at approximately the same figure.

The distribution of the dates of first head in the parental races and in the F_3 hybrids for 1916 is shown in the following table:

TABLE 11
Date of first head in $(3 \times 34) F_3$, 1916.

	March								April											
	17 18	19 20	21 22	23 24	25 26	27 28	29 30	31 1	2 3	4 5	6 7	8 9	10 11	12 13	14 15	16 17	18 19	20 21	22 23	
Pure No. 3.....										1	1		14	87	138	1				
(3 × 34) F ₃		12	17	43	139	415	761	675	597	842	391	195	157	30	1103	21	17	1	1	
Pure No. 34.....	1	30	41	56	23	74	17	1												

Means of cultures.

Pure No. 3.....													3	2					
$(3 \times 34) F_3$					1	12	9	14	15	21	9	6	10	7	8				
Pure No. 34.....			1	1	2	1													

It is interesting to note here that no hybrid plant was earlier than the earliest individual of the early culture and that there were only 19 later than the latest of the late parent. Again considered as cultures, the means of the hybrid cultures all fall within the limits set by the extreme means of the parental variety cultures. Here recombination does not seem to have extended the variability definitely beyond the limits of the parents.

Tables 12 and 13 show the segregation of the F_3 to be just as marked in this cross as in the cross already discussed. The greater variability of the intermediate classes is also quite evident. This fact taken in connection with the fact that there was no indication of partial sterility among the hybrids seems significant. It is exactly what should be expected if the segregation of the F_2 plants and F_3 cultures were due to recombination. This should be contrasted with the absence of greater variability of intermediates in the semi-sterile hybrids of the bread wheat—macaroni wheat crosses.

TABLE 12

Date of first head in (3×34) F_3 , 1916. Distribution based upon dates of first head of the selected F_2 parents.

F ₃ individuals																								
Number of cultures	March												April											
	9 10	11 12	13 14	15 16	17 18	19 20	21 22	23 24	25 26	27 28	29 30	31 1	2 3	4 5	6 7	8 9	10 11	12 13	14 15	16 17	18 19	20 21	22 23	
1	+						1	2	1		13 16	11 11	14	5					3					
2				+						56 172	16 47			22										
7					+	2	6	37	16	172	47	30	3	21										
13						+		1	16	72	153	103	30	158	10	22	4	3	56	2	1			
38						10	10	3	104	91	481	396	248	214	59	45	26		101	6				
13							+			2	3	49	85	121	170	37	26	15	12	84		5		
14								+		11	1	37	57	195	151	36	31	14	124	4	8	1		
21										+	1	13	24	57	134	56	80	1	619	2				
3											+					14	1		125	1			1	

+= Selected F_2 parents.

○ = Mean of group.

		Means of F ₃ cultures																	
Number of cultures	Average σ of F ₃ cultures	March				April													
		25 26	27 28	29 30	31 1	2 3	4 5	6 7	8 9	10 11	12 13	14 15	16 17	18 19	20 21	22 23			
1	6.30				1														
2	1.47			1	1														
7	2.01	1	4	1	1														
13	3.79		1	3	1	3	5												
38	3.46		6	5	9	9	7	1		1									
13	4.02				1	3	4	3	1	1									
14	3.82					1	3	4	3	3									
21	2.46						1	1	2	5		6	6						
3	2.03										1	2							

TABLE 12 (continued)

Date of first head in (3×34) F_2 , 1916. Distribution based upon dates of first head of the selected F_2 parents.

Standard deviations of F_2 cultures.

Number of cultures	.25	.75	1.25	1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75	6.25	6.75
1													1	
2	1					1								
7			1	3	1	2								
13			1	1	2	1			5		1		2	
38			1	5	7	4	3	4	2	5	4	2		
13				2	1			2	2	1	3	1		
14				1		3	1	2	4	1	1	1		
21	1	2	3	1	4	3	3	2	1	1				
3			1	1			1							

TABLE 13

Date of first head in (3×34) F_2 , 1916. Distribution based upon means of F_2 cultures.

F_2 individuals

Number of cultures	March										April												
	9 10	11 12	13 14	15 16	17 18	19 20	21 22	23 24	25 26	27 28	29 30	31 1	2 3	4 5	6 7	8 9	10 11	12 13	14 15	16 17	18 19	20 21	22 23
1						2	4	24	○	15	1												
12						8	9	16	79	200	214	38	1	1	1								
9							3	30	72	195	108	13	6	1					1				
14					1	1	2	20	40	157	222	101	92	7		3		17					
16								9	55	116	136	108	221	37	18	6	2	59	2				
20					1		1	1	20	78	136	192	291	○	58	24	31	2	101	8	1		
9									13	1	27	69	150	71	○	7	19	5	79	1			
6										1	1	9	52	84	45	26	7	52	3	5			
10												4	27	112	58	46	13	213	6	1			
7													2	19	40	24	1	248					
8															3	8	2	352	2	1		1	

○ = mean of group.

TABLE 13 (continued)

Date of first head in (3 × 34) F₂, 1916. Distribution based upon means of F₂ cultures.

Number of cultures	Average σ of F ₂ cultures	Selected F ₂ parents															
		March												April			
		9 10	11 12	13 14	15 16	17 18	19 20	21 22	23 24	25 26	27 28	29 30	31 1	2 3			
1	2.55					1											
12	2.05				1	4	1	6									
9	2.05					1	3	5									
14	3.35	1			1	1	1	9	1								
16	4.18						3	9	3		1						
20	4.32						5	7	4		1						
9	4.18							1	3	4	1						
6	3.74								1	3	2						
10	3.63							1	1	3	5						
7	2.53										6	1					
8	1.21										6	2					

Standard deviations of F₂ cultures.

Number of cultures	.25	.75	1.25	1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75	6.25	6.75
1			1	3	3	1								
12	1		2	2	4	3	1							
9				4	1	3	1	1	1	1	1	1	1	
14				1	1	1	1	1	5	2	2	1	1	
16				2	1			1	4	3	4	2	2	
20		1						1	2	2	2			
9						2		2	1	2	2			
6						1	1	1	2	1				
10							4	4	2					
7				1	2	4								
8	1	1	4	1	1									

Summary; date of first head

In both crosses the parents had wide differences in heading dates and the averages of the F₂ and F₃ were in every case intermediate and nearer to the late parent. The range of the individual hybrid plants in no case extended significantly beyond the range of the early parent toward extreme precocity of heading. Toward the late extreme, however, in the macaroni—bread wheat crosses, there was a long extension of the range, much beyond that of the late parent. As a matter of fact many plants never headed, but remained as dark green, grass-like tufts until they were killed by the heat and dryness of the summer. Among the bread wheat crosses the extension of the range of date of first head beyond the ex-

treme of the late parent was never marked and could, in fact, be accounted for by the normal extension of the curve due to greater numbers.

The same observations made above with regard to the relation of the means of the hybrid populations to their parental means, apply also to the distribution of the means of the hybrid cultures, as compared with their parents, in the F_2 . In the F_3 , however, the matter was somewhat different. In the macaroni—bread wheat cross there were 3 cultures whose average dates of first head were earlier than the earliest parental average and there were altogether 19 cultures averaging later than the latest parental average. Since there were 230 cultures concerned, 8.2 percent are thus seen to lie outside of the parental range. In the bread wheat cross, on the other hand, there was no case where the average of a hybrid culture was outside the range of averages for the parental varieties. As regards individuals in the F_2 the parental types were abundantly recovered in every case. As regards means of F_3 cultures (a better criterion of the genetic constitution of the F_2 plants) the parental types were also recovered in all cases.

In all cases where more than one culture was involved the standard deviations of the population were greater than the average of the standard deviations of the cultures taken separately and in all cases the standard deviations of the hybrids¹ were greater than those of either parent both as regards that of the populations and the averages of the cultures taken separately.

In comparing the standard deviations of the hybrid F_3 populations with their respective F_2 parental populations we may note the following observations: (1) the standard deviation of F_3 populations are so dependent upon the range of F_2 parents chosen, that conclusions drawn from the calculation of this constant should be carefully guarded. The standard deviation of the F_3 population of both crosses was greater than that of the F_2 population. Since heading time appears to be imperfectly dominant in these hybrids, the number of intermediate types will tend to be reduced as the population approaches homozygosity. If therefore we assume a Mendelian inheritance, whenever the selected F_2 parents practically cover the range of distribution of the F_2 population and form a random sample thereof, we would expect the F_3 population to have a higher standard deviation than the F_2 population.

When we come to compare the average variability (here measured by standard deviation) of the F_3 cultures taken separately with the average

¹ It should be remembered that the F_1 is not here included.

variability of the F_2 cultures we are not hampered in our conclusions, to so large an extent as mentioned above in comparing the variability of the F_2 and F_3 populations. With a Mendelian interpretation there is no genetic reason why any F_3 culture should be significantly more variable than the most variable F_2 culture. Moreover, the average variability of the F_3 should be equal to or less than that of the F_2 , whatever the mode of selection. We may now observe as follows: (1) In the macaroni—bread wheat cross, 1×35 , the average variability of the F_3 cultures was significantly below that of the F_2 cultures. (2) In the bread wheat cross some complications arose. The average standard deviations of the F_2 and F_3 cultures of the 3×34 were the same (2.95). This, however, cannot be assumed as evidence of a lack of progress toward homozygosity, for the following reasons: It will be observed that the variability of pure race No. 34 was strongly increased in 1916 over 1915 (2.17 and 1.75, respectively) although all 5 of the cultures grown in 1916 came from the 1 culture grown in 1915, which in turn came from a single plant in 1914. Perhaps the same factors which caused this increase in the variability of the pure line No. 34 were also able to increase the variability of the hybrid cultures which were grown from No. 34 as one parent and that this influence upon the variability was sufficient to offset that of increasing homozygosity and thus maintain the variability for the two seasons at the same figure.

The strongly fluctuating nature of the variability of date of first head is shown by a study of the distribution of the standard deviations of the F_2 . In every case the range of distribution of the standard deviations of the F_2 overlapped the range for one or both parents. This could be explained by assuming a partial-blending inheritance and assuming that in some F_1 plants the blend was more complete than in others. If this were true the F_3 cultures grown from these low-variable F_2 cultures should also show a low variability. The results are given in table 14.

TABLE 14

Number of F_2 cultures as little variable as one parent	Number of F_3 cultures arising from these	Average σ of these F_3 cultures	Number of F_2 cultures more variable than either parent	Number of F_3 cultures arising from these	Average σ of these cultures
22	148	3.12	21	194	3.05

It is thus seen that the low-variable F_2 cultures gave rise to the higher-variable F_3 cultures. This is what would be expected upon a Mendelian

interpretation if we assumed that the low variability of the F_2 cultures in question were so because but few of the extreme combinations chanced to occur. It must be admitted however that the difference shown is not large enough to be significant. We may therefore safely conclude that the differences in standard deviations of the F_2 cultures were wholly fortuitous and without genetic significance.

In the F_3 generation, in all cases, cultures occurred with as low variability as that of the parents, i.e., there were cultures which, insofar as variability is concerned, appeared as nearly homozygous as the pure lines.

With a Mendelian interpretation we are accustomed to expect those F_2 plants which take a position relative to the parents similar to that occupied by the mode of the F_1 , to give rise to F_3 cultures which are more variable than the F_2 plants otherwise located. In the macaroni—bread wheat crosses we are not able to observe any relation of this kind. This fact, however, does not argue the absence of Mendelian segregation for the following reasons: The macaroni and bread wheats here crossed, differ in so many genetic factors that there is an extremely wide range in the products of their recombination. Many of these recombinations are so radical and unbalanced that they are no longer automatic. Hence there is a high percentage of sterility in the F_2 and later generations. Such sterility may have the effect of flattening the distribution curve of the F_2 or perhaps even limiting it to one end or the middle or even the extremes of a curve which would be formed by all of the recombination possibilities. As already pointed out many of the F_2 plants never got beyond the rosette stage and many plants which made a robust vegetative growth were completely sterile. The study of sterility in these crosses will be reserved for a future paper. In circumstances such as these it is apparent that there may occur very little difference in the heterozygosity, hence variability, of the cultures from individuals selected from either the middle or extremes of the fertile F_2 of such a population. In the 3×34 cross there is a very apparent greater variability of the cultures arising from the modal F_2 plants (see tables 12 and 13). It should be noted that here there was complete fertility and the F_2 selections covered nearly the whole of the range of the F_2 population. A glance at tables 6 and 12, where the F_3 individuals are grouped with reference to the heading date of the F_2 parents, yields abundant evidence that some sort of segregation has occurred. The F_2 plants were not alike genetically. All of the phenomena observed can be explained by assuming that heading date is governed by three or more Mendelizing unit factors. No attempt has been made to determine the number

of factors in any case but the fact that many of the intermediate groups (see tables 6 and 13) show cultures with low variability would indicate that the number of factors concerned was rather large, thus providing the possibility of securing several genetically different but still homozygous types.

HEIGHT

Macaroni—bread wheat crosses. Algerian macaroni (No. 1) × Sonora (No. 35)

In this study all height measurements were made from the ground to the top of the highest head (not including the awns). Lengths were taken to the nearest centimeter and expressed in the summaries to the nearest five centimeters. No pure No. 35 was grown in 1914 which was comparable with the pure No. 1 and the $(1 \times 35)F_1$. The No. 1 grown in 1914 was not a single pure line but was from seed of several different mother plants of this variety. A summary of the results for 1914 is shown in table 15.

TABLE 15
Heights in centimeters in $(1 \times 35) F_1$, 1914.

	Number of plants	70 79	80 89	90 99	100 109	110 119	120 129	130 139	140 149	150 159	160 169	Aver- age	C.V.
Pure No. 1.....	151	1		1	3	9	26	43	49	18	1	134	10.0
$(1 \times 35) F_1$	39				1		1	4	8	21	4	147	8.0

The F_1 was taller but no more variable than the parent given. Thirty-eight of these hybrid plants gave rise to hybrid cultures in 1915. The results are summarized in table 16.

TABLE 16
Heights in $(1 \times 35) F_2$, 1915.

	Number of cultures	Number of individuals	Average height	Coefficient of variation	
				of population	of separate cultures
Pure No. 1.....	9	648	147	8.5	6.7
$(1 \times 35) F_2$	38	2535	122	19.6	19.0
Pure No. 35.....	3	166	128	11.1	6.4

Distribution of the coefficients of variation of cultures.

	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Pure No. 1.....		3	2	2	1					1											
$(1 \times 35) F_2$													2	8	3	9	8	4	2	1	1
Pure No. 35.....	1		1			1															

It should be noted here that, whereas the F_1 was taller than No. 1, the tall parent, the average of F_2 (where all of the F_1 was planted) was lower than either parent. The high sterility of the F_2 plants has already been noted. As usual the hybrids were more variable than either parent. It should also be noted that the F_2 hybrids were much more variable than the F_1 .

Table 17 gives the distribution of the populations and means of both parents and the F_2 hybrids as regards height.

TABLE 17
Heights in centimeters in (1×35) F_2 , 1915.

	Distribution of individual heights																		Distribution of means of cultures				
	30 39	40 49	50 59	60 69	70 79	80 89	90 99	100 109	110 119	120 129	130 139	140 149	150 159	160 169	170 179	180 189	190 199	200 209	210 219	220 229	230 239	240 249	250 259
Pure No. 1						1	4	4	4	25	89	155	217	139	10						2	3	4
(1×35) F_2	5	2	18	29	51	104	178	226	311	409	447	399	248	94	11	3	4	30	4		1		
Pure No. 35								8	25	29	52	38	13	1				1	1	1			

Only three of the hybrid plants were taller than the tallest individuals of the tall parent, but there were 95 lower than the lowest individual of either parent. No hybrid culture averaged as tall as the highest average for the low parent, but 4 cultures averaged lower than the lowest average of either parent. All recombinations so far obtained appear therefore to be less vigorous than the parental races. Since the F_1 plants showed considerable range in height, it would be interesting to know whether this was inherited to any degree in F_2 , i.e., was the range in F_1 due solely to modification or were these differences partly genetic? Table 18 shows the F_2 cultures grouped according to the parental height. The class in which the parental height fell is marked +, and the mean of the population arising from such parents is marked O.

While the last class is 8 cm higher than the first class, considering the small number of races in each, this difference is not above the probable error. We may therefore safely conclude that for all practical purposes the F_1 plants were uniform genetically.

Two hundred and thirty of the F_2 plants were selected for planting in the fall of 1915 and gave rise to hybrid cultures which were measured just before ripening in 1916. For comparison 7 pure cultures of No. 1 and 5 pure cultures of No. 35 were grown. The first summary of results follow.

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TABLE 18
Heights in centimeters in (1 X 35) F₂, 1915.

Number of cultures	Height of parent	Average height of offspring	Number of individuals	Heights in centimeters in (1 X 35) F ₂ , 1915.																								
				30	39	40	49	50	59	60	69	70	79	80	89	90	99	100	110	120	130	140	150	160	170	180	190	
1	120 129	118	37							3					2	6			0	+	6	10	3	3				
4	130 139	123	228	1				3				8		6	18			20	25	34	48	+	38	21	6			
8	140 149	122	485	2				2	7	3	20			39	40			57	68	0	87	+	78	51	27	2	2	
21	150 159	123	1488	1	2	11	16	37	65	98	143	199	249	253	223	139	46	+	5	1								
4	160 169	126	297	1				2	3	3	11	17	23	26	52	49	34	15	4									

TABLE 19
Height in centimeters in $(1 \times 35) F_2$, 1916.

	Number of cultures	Number of individuals	Average height	Coefficient of variation of the population	Average C.V. of separate cultures
Pure No. 1....	7	344	137	8.4	6.6
$(1 \times 35) F_2$...	230	10084	118	20.3	15.4
Pure No. 35...	5	246	123	7.1	6.3

Distribution of coefficients of variation in $(1 \times 35) F_2$, 1916.

	3 4	5 6	7 8	9 10	11 12	13 14	15 16	17 18	19 20	21 22	23 24	25 26	27 28	29 30	31 32	33 34	35 36	37 38	39 40	41 42	43 44	45 46	47 48	49 50	51 52
Pure No. 1...	4	2	1																						
$(1 \times 35) F_2$...	6	15	24	35	38	28	21	20	19	11	3	4	3	1				1							1
Pure No. 35...	1	2	1	1																					

As usual it may be observed that the pure races are less variable than the hybrids and that the average coefficient of variation of the cultures is smaller than those of the populations. It should be further noted that the average coefficient of variation of the F_2 hybrid cultures is smaller than that of the F_1 . This is to be expected in the case of increasing homozygosity.

Table 20 shows the distribution of the populations in 1916.

TABLE 20
Heights in centimeters in $(1 \times 35) F_2$, 1916.

	10 19	20 29	30 39	40 49	50 59	60 69	70 79	80 89	90 99	100 109	110 119	120 129	130 139	140 149	150 159	160 169	170 179	180 189	190 199
Pure No. 1....								2	2	1	9	83	114	95	33	6			
$(1 \times 35) F_2$...	1	12	15	62	127	217	404	496	862	1335	1723	1757	1435	1077	471	75	8	5	2
Pure No. 35...							1	3	1	10	72	141	17	1					

Distribution of means.

Pure No. 1....												2	2	3					
$(1 \times 35) F_2$...						1	2	13	21	49	53	42	36	12	1				
Pure No. 35...											2	3							

Only 15 hybrid plants were taller than the tallest individuals of the tall culture. Considering the large number of hybrids in comparison with the number of No. 1, these few taller plants are without significance. At the other end of the scale, however, we find 474 plants lower than the lowest of the lower parent. Considering means we also note with interest that there were 86 hybrid cultures averaging lower than the lowest average for the low parent and one hybrid culture averaging lower than the lowest individual of the low parent.

TABLE 21
Heights in centimeters in (1 × 35) F₂, 1916.

Number of cultures	Arrangement of F ₂ individuals grouped according to F ₂ parents																		
	10 19	20 29	30 39	40 49	50 59	60 69	70 79	80 89	90 99	100 109	110 119	120 129	130 139	140 149	150 159	160 169	170 179	180 189	190 199
3				9	12	13	11	9	17	27	29	5							
8		1	2	3	5	11	32	30	67	83	52	21	5	4	1				
12			1	2	11	13	21	35	65	96	123	62	31	22	3	1			
24		7	1	10	21	32	54	72	160	226	240	128	71	21	6	1			
35		1		12	24	33	79	106	144	250	326	304	168	56	16	2			
55	1	2	7	20	31	61	99	107	174	310	473	507	328	223	89	6	2		
48			4	5	12	36	62	76	124	186	271	428	423	351	138	15		2	2
40		1		5	5	21	38	47	92	141	178	282	385	354	174	41	5	2	
4				1	4	2	8	11	11	11	15	19	21	39	31	3	1		
1													1	27	17	5	+		

Number of cultures	Distribution of means of F ₂ cultures										Average coefficient of variation
	60 69	70 79	80 89	90 99	100 109	110 119	120 129	130 139	140 149	150 159	
3	1			2							16
8			1	2	4	1					18
12			3		5	3		1			16
24		1	2	6	7	8					17
35			3	4	11	10	5	2			15
55		1	3	4	11	17	11	6	2		16
48			1	4	5	7	14	14	3		14
40				1	3	8	10	13	5		14
4					1	1			2		18
1									1		5

Number of cultures	Distribution of coefficients of variation																									
	1 2	3 4	5 6	7 8	9 10	11 12	13 14	15 16	17 18	19 20	21 22	23 24	25 26	27 28	29 30	31 32	33 34	35 36	37 38	39 40	41 42	43 44	45 46	47 48	49 50	51 52
3						2						1														
8						1	1	2	1	1	1	1														
12				1	2	1	2	2	1		2									1						
24			1	1	2	3	4	2	4	3	1			2												1
35				2	4	4	5	8	2	2	6		2													
55				2	9	10	9	3	5	4	4	3	1	2	2	1										
48				2	3	7	8	6	6	4	7	3	1			1										
40				1	5	1	6	11	3	5	3	1	4													
4						1			1		1	1														
1				1																						

TABLE 22

[illegible]

Table 21 shows the height of the F_3 plants grouped according to their F_2 parents, the means of the F_3 cultures and the coefficients of variation of these cultures, respectively, making up each population group. Table 22 shows the height of the F_3 plants grouped according to the means of the F_3 cultures, the heights of the parents giving rise to these groups and the standard deviations and coefficients of variation of the F_3 cultures, respectively. It should be noted in table 21 that, while there was considerable regression toward the mean, there was a nearly uniform correlation between the height of the F_2 parent and the F_3 offspring. By comparing table 21 with table 20 it will be observed that the distribution of the means in any group of hybrids is no wider than the range of variation of the individuals in either of the parental varieties. Observing the averages and distribution of the coefficients of variation we note an irregular but yet fairly definite lessening of variability in the taller groups.

Again comparing table 22 with table 20 we note that for any F_3 group (in table 22) the distribution of the parents was not wider than the distribution of the individuals of the parental varieties. The differences in the heights of the individuals of these parental groups (which gave rise to cultures having the same mean) could therefore be assumed to be environmental modifications of plants of the same or equivalent heredity so far as height is concerned.

The column showing the average coefficient of variation and the distribution of these constants in table 22 shows a very decided decrease in variability of those cultures which have high means.

One conclusion stands out prominently from these tables. The factors for height were not uniform in the F_2 plants. Recombination had occurred so that on the average (i.e., excluding environmental modifications), tall parents gave rise to tall offspring and the grading of the parents into a series of ascending heights resulted in a slightly less marked but still regularly ascending series of offspring groups. The completeness of this series indicates that the number of factors was large.

Algerian macaroni (No. 1) \times Algerian red bread (No. 3)

In 1914, 151 plants of pure No. 1 and six plants of pure No. 3 together with 5 plants of $(1 \times 3) F_1$ were measured for height.

The following table shows the distribution of the heights of these plants and their means. Except for the pure No. 1, the numbers were too small for the calculation of the standard deviations with any degree of accuracy.

TABLE 23
Heights in centimeters in the $(1 \times 3) F_1$, 1914.

	Number of plants	70 79	80 89	90 99	100 109	110 119	120 129	130 139	140 149	150 159	160 169	Mean
Pure No. 1.....	151	1		1	3	9	26	43	49	18	1	134
$(1 \times 3) F_1$	5				2			1	2			124
Pure No. 3.....	6				2	1	2	1				118

The numbers are too small to give results of any particular significance, but it may be noted that the range of the F_1 hybrids lies within the range of the most variable parent and that the mean of the hybrids lies between the means of the two parent cultures.

The 5 F_1 hybrid plants gave rise to 5 hybrid F_2 cultures in 1915. For comparison in the same year 9 cultures of No. 1 and 1 culture of No. 3 were available. Table 24 gives a summary of the results.

TABLE 24
Heights in the $(1 \times 3) F_2$, 1915.

Culture	Number of cultures	Number of individuals	Average height	Coefficient of variation of the population	Average C.V. of the separate cultures
Pure No. 1....	9	648	147	8.5	6.7
$(1 \times 3) F_2$	5	406	118	21.1	20.4
Pure No. 3....	1	42	146	4.2	4.2

Distribution of coefficients of variation.

	3 4	5 6	7 8	9 10	11 12	13 14	15 16	17 18	19 20	21 22	23 24	25 26	27 28	29 30	31 32
Pure No. 1...		5	3			1									
$(1 \times 3) F_2$..				1			1		1			1			1
Pure No. 3...	1														

Whereas the F_1 hybrids were intermediate between the parent races, the F_2 averaged lower than either, the two parent races being of practically equal height. The variability of the hybrids was strikingly higher than that of the parental cultures.

Table 25 gives the distribution of the populations and the means of both parents and the F_2 hybrids as regards height.

None of the hybrid plants was taller than the tallest individual of the parental cultures but there were 29 lower than the lowest individual of the parents. It is striking that all of the means of the hybrid cultures save one were lower than the lowest parental mean. All recombinations, therefore, appear to be less vigorous than the parental cultures.

TABLE 25
Heights in centimeters in the (1×3) F_2 , 1915.

	40 49	50 59	60 69	70 79	80 89	90 99	100 109	110 119	120 129	130 139	140 149	150 159	160 169	170 179
Pure No. 1....					1	4	4	4	25	89	155	217	139	10
(1×3) F_2	7	5	8	9	17	27	39	57	61	87	34	27	8	
Pure No. 3....										3	18	21		

Distribution of means of cultures.

Pure No. 1....										2	3	4		
(1×3) F_2							2	1	1	1				
Pure No. 3....											1			

Table 26 shows the F_2 cultures grouped according to the height of their respective F_1 parents. The class in which the parental height fell is marked + and the means of the population arising from such parents are marked O.

TABLE 26
Heights in centimeters in the (1×35) F_2 , 1915.

Number of cultures	Height of parent	Average height of offspring	Number of individuals	30	40	50	60	70	80	90	100	110	120	130	140	150	160
				39	49	59	69	79	89	99	109	119	129	139	149	159	169
2	100 109	105	82		2	4	5	4	9	10	O+	9	10	12	9	6	2
1	130 139	110	54		3	1		2	4	3		O		+			
											5	9	9	13	3	2	
2	140 149	126	270		2		3	3	4	14	25	38	40	65	45	23	8

Although the range of each of these groups is practically the same, the distinct correlation between the height of parent and height of offspring cannot be disregarded. This would indicate that one or the other of the parental stocks was not pure as regards the factors influencing height and that the F_1 plants were, therefore, not all equivalent genetically in this respect. In order, therefore, to avoid complications, the subsequent discussion of this cross will be based upon the product of a single F_1 plant (145 cm high) in 1914 from which a culture (No. 32-1) was grown in 1915, of which the following data may be given:

TABLE 27
Heights in centimeters in the (1×35) F_2 , 1915.

Culture	Height of parent	Average height of offspring	Number of individuals	Average C.V.	Distribution of heights of individuals									
					70	80	90	100	110	120	130	140	150	160
32-1	145	130	71	15	79	89	99	109	119	129	139	149	159	169
					1	1	4	10	7	11	9	13	10	5

From this culture 40 plants were selected as parents in 1915-'16. A first summary of the results may be given as follows:

TABLE 28
Heights in centimeters in $(1 \times 3)F_2$, 1916.

Cultures	Number of cultures	Number of individuals	Average height	Coefficient of variation of the population	Average C.V. of separate cultures
Pure No. 1....	7	342	137	8.5	6.6
$(1 \times 3) F_2$	40	1758	123	20.6	14.2
Pure No. 3....	5	243	133	8.0	6.6

Distribution of coefficients of variation

Cultures	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39	41	43
	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44
Pure No. 1.....		4	2	1																	
$(1 \times 3) F_2$	1	6	3	5	5	3	3	2	5	2	2	1				1					1
Pure No. 3.....	2	1	1		1																

Again we perceive that the averages of the coefficients of variation of the cultures are less than the coefficients of variation of their respective populations, and that the pure lines are less variable than the hybrids. The average variability of the F_2 is markedly less than that of the cultures in F_2 .

Table 29 gives the distribution of the populations and means of both the hybrid and parental cultures.

TABLE 29
Heights in centimeters in $(1 \times 3)F_2$, 1916.

	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170
	39	49	59	69	79	89	99	109	119	129	139	149	159	169	179
Pure No. 1.....						1	1	1	1	11	91	123	84	26	3
$(1 \times 3) F_2$	8	6	21	21	67	73	100	157	244	274	252	320	170	36	8
Pure No. 3.....		1			1			1	10	68	108	48	6		

Distribution of means.

Pure No. 1.....										2	2	3			
$(1 \times 3) F_2$						2	4	5	9	6	3	9	2		
Pure No. 3.....										3	2				

Observing tables 28 and 29 it is evident that on the average, height-vigor in the F_2 hybrids was again less than for the two parental cultures but that there were two hybrid cultures taller than the tallest aver-

age for the taller parent. On the other hand 20 hybrid cultures were lower than the lowest average of the low parent.

Table 30 shows a fairly uniform correlation between the height of the selected F_2 parent and the average height of its F_3 offspring. Table 31 exhibits rather strikingly the fact that the taller F_3 cultures are much less variable than those which averaged lower. Now if one will compare the distribution of the selected F_2 parents (table 30) with the total F_2 population as shown in table 25, it will be observed that the selections just cover the upper half of the range. As regards the variability of the F_3 , therefore, table 31 and the accompanying column of average coefficients of variation might be assumed to represent only a half curve. The low selections were therefore really intermediate F_2 individuals. The higher variability of these lower F_3 cultures, and the very evident decline in variability as we approach the taller, real, extreme, can be interpreted as being in accord with the idea of hybrid recombination of height factors with the intermediate forms most heterozygous and hence more variable.

TABLE 30
Heights in centimeters in $(1 \times 3)F_3$, 1916.

Number of cultures	10 19	20 29	30 39	40 49	50 59	60 69	70 79	80 89	90 99	100 109	110 119	120 129	130 139	140 149	150 159	160 169	170 179
2					1		4	7	13	+	13	31	8	1			
3			2	1	6	4	12	17	16	19	+	20	10	3	4		
7			1	3	4	5	19	16	22	42	87	+	67	36	19	2	
9			3	2	6	7	20	17	21	47	66	+	57	45	10	3	1
11			2		2	5	8	9	18	27	32	49	80	+	147	89	27
6					1		4	7	9	10	22	30	54	72	+	48	1
2									1	1	4	7	14	32	21	+	5

+, Selected F_2 parents.

O, Means of F_3 groups.

Number of cultures	Distribution of means of F_3 cultures									Average coefficient of variation
	70	80	90	100	110	120	130	140	150	
	79	89	99	109	119	129	139	149	159	
2				1	1					15
3		1	1	1						25
7		1		1	3	2				16
9			2	1	2	3		1		17
11			1	1	1		3	3	2	11
6					2	1		3		12
2								2		10

TABLE 30 (continued)
Heights in centimeters in (1 × 3) F₃, 1916.

Number of cultures	Distribution of coefficients of variation in F ₃ cultures																					
	3 4	5 6	7 8	9 10	11 12	13 14	15 16	17 18	19 20	21 22	23 24	25 26	27 28	29 30	31 32	33 34	35 36	37 38	39 40	41 42	43 44	
2					I			I														
3						I			I												I	
7				I	I		I	I	I	I	I											
9				I	2		I	I	I	I	I					I						
11	I	3	I		4			I					I									
6		3						I		2												
2				2																		

TABLE 31
Heights in centimeters in (1 × 3) F₃, 1916.

Number of cultures	Distribution of F ₃ Individuals																
	10 19	20 29	30 39	40 49	50 59	60 69	70 79	80 89	90 99	100 109	110 119	120 129	130 139	140 149	150 159	160 169	170 179
2			1	2	4	5	20	20	21	9	5	4					
4			4	3	11	10	17	11	20	18	17	24	6	7	1		1
5			2		1	2	10	18	25	49	55	32	10	2			
9					4	2	10	15	23	55	114	109	46	13	5		
6			1	1	1	2	8	6	8	20	38	73	66	38	5		
3							2	3	2	3	8	17	43	49	13	1	
9									1	2	7	15	77	194	108	10	
2										1			4	17	38	25	7

O, Means of F₃ groups.

Number of cultures	Distribution of F ₂ parents							Average coefficient of variation
	100 109	110 119	120 129	130 139	140 149	150 159	160 169	
2			1					22
4		1		2	1			31
5	1	1	1	1	1			16
9	1		3	2	1	2		15
6			2	3		1		15
3					3			12
9				1	3	3	2	6
2					2			7

TABLE 31 (continued)
Heights in centimeters in (1 × 3) F₂, 1916.

Number of cultures	Distribution of coefficients of variation in F ₂ cultures																						
	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39	41	43		
	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44		
2									1		1												
4										1			1				1				1		
5							1	2	2														
9				1	1	2	1			2	2												
6					2		1	1		1		1											
3						3																	
9	1	5	1	2																			
2		1	1																				

Height in bread wheat crosses, 3 × 35

No pure No. 35 was grown in 1914 for comparison with the pure No. 3 and the F₁ hybrids of 3 × 35. The following table summarizes the data for the pure No. 3 (6 plants, not a pedigree line) and the (3 × 35) F₁ hybrids.

TABLE 32
Heights in centimeters in (3 × 35) F₁, 1914.

Culture	Number of plants	Average height	Distribution of heights of individuals							
			100 109	110 119	120 129	130 139	140 149	150 159	160 169	170 179
Pure No. 3	6	118	2	1	2	1				
(3 × 35) F ₁	18	142			1	5	8	3	1	

The hybrids are thus seen to be taller than the pure No. 3 and the range is slightly greater, but not more than would be expected with the larger number of individuals grown, i.e., one could not infer that the hybrids were more variable than the pure race.

Each of the 18 F₁ plants gave rise to an F₂ culture in 1915. For comparison 3 cultures of No. 35 and one of No. 3 are available. Table 33 summarizes the results for 1915.

TABLE 33
Heights in centimeters in (3 × 35) F₂, 1915.

Culture	Number of cultures	Number plants	Average height	Coefficient of variation of the population	Average C.V. of separate cultures	Distribution of C.V.			
						3 4	5 6	7 8	9 10
Pure No. 3	1	42	146	4.2	4.2	1			
(3 × 35) F ₂	18	1611	148	7.4	6.0		15	1	2
Pure No. 35	3	166	128	11.1	6.4	1	1		1

It is here interesting to note that the hybrids are somewhat taller than the tall parent.

Table 34 gives the distribution within the populations of F_2 hybrids and parental races. In the hybrids, the cultures are arranged in groups with regard to the height of their F_1 parents.

TABLE 34
Heights in centimeters in $(3 \times 35) F_2$, 1916.

	Number of cultures	Parental height	60 69	70 79	80 89	90 99	100 109	110 119	120 129	130 139	140 149	150 159	160 169	170 179
Pure No. 3	1									3	18	21		
$(3 \times 35) F_2$	1	120 120				1		1	+	4	8	0	40	2
"	5	130 139	1				1		14	+	46	144	0	169
"	8	140 149				1	2	3	18	80	+	0	236	65
"	3	150 159				2	1	1	5	12	57	+	0	105
"	1	160 169							2	5	13	0	52	+
$(3 \times 35) F_2$ Totals	18		1			4	4	5	43	154	0	508	663	235
Pure No. 35	1						8	25	29	52	38	13	1	

Distribution of means of cultures.

Pure No. 3											1			
$(3 \times 35) F_2$										1	1	6	11	
Pure No. 35									1	1	1			

+, Selected F_1 parent.

0, Mean of group.

No appreciable correlation between the height of the F_1 parent and the average of the F_2 offspring is apparent. We may therefore consider that so far as the height factors are concerned, the F_1 plants were all equivalent. The range of distribution of the hybrid population slightly exceeded that of the most variable parent in both directions but no more than would be expected considering the larger number of plants grown.

From the above F_2 hybrids 80 selections were made for growing in 1915-16. These ranged from 118 to 173 cm high, thus covering all of the upper but not quite all of the lower end of the range of the F_2 . For comparison with these, 5 cultures of each of Nos. 3 and 35 were grown. A first summary of the results are shown in table 35.

TABLE 35
Heights in centimeters in $(3 \times 35) F_2$, 1916.

Culture	Number of cultures	Number of individuals	Average height	Coefficient of variation of the population	Average C.V. of separate cultures
Pure No. 3....	5	243	133	8.0	6.6
$(3 \times 35) F_2$...	80	3849	143	8.4	6.3
Pure No. 35...	5	246	123	7.2	6.3

Distribution of coefficients of variation.

	3 4	5 6	7 8	9 10	11 12	13 14
Pure No. 3.....	2	1	1		1	
$(3 \times 35) F_2$	8	43	21	4	3	1
Pure No. 35.....	1	2	1	1		

It should here be noted that the average height of the hybrids is again greater than that of the taller parent and that there is no diminution in the variability of the F_3 from the F_2 . Moreover, the hybrids are no more variable than the pure races.

Table 36 gives the distribution of the populations of the hybrids and their parental races as well as the distributions of the means of the cultures of each.

TABLE 36
Heights in centimeters in $(3 \times 35) F_2$, 1916.

	Distribution of individuals															Distribution of means of cultures				
	40 49	50 59	60 69	70 79	80 89	90 99	100 109	110 119	120 129	130 139	140 149	150 159	160 169	170 179	110 119	120 129	130 139	140 149	150 159	
Pure No. 3..	1			1			1	10	68	108	48	6				3	2			
(3 × 35) F ₂ .				1	3	1	37	164	519	1045	1350	611	104	14		12	26	34	8	
Pure No. 35.				1	3	1	10	72	141	17	1				2	3				

That we should here have 42 hybrid cultures (slightly more than half) whose average heights were higher than the highest average for the tall parent is somewhat surprising. Especially is this so when we reflect that the variability of the hybrids is no greater than that of the pure lines.

From table 37 we observe that the regression of the offspring of extreme selections is quite strong, but it is not complete. The difference between the means of the offspring of selected extremes is greater than

between the means of the parental races (compare table 35). Comparing the distribution of selected F_2 parents forming the groups in table 38 with the distribution of the individuals of their parental varieties in table 34, we will note that they are not more widely distributed. They can therefore be assumed to be environic modifications of individuals representing equivalent genetic combinations so far as height is concerned. There was a fairly well marked decrease in the variability of the taller cultures.

TABLE 37
Heights in centimeters in (3 × 35) F_2 , 1916.

Number of cultures	Arrangement of F_2 individuals grouped according to F_2 parents										
	69 78	79 88	89 98	99 108	109 118	119 128	129 138	139 148	149 158	159 168	169 178
1				5	+	0	5	15	3		
2				2	13	+	0	21	24	23	2
9	1	2		7	43	110	0+	79	96	73	19
20			1	15	33	188	265	314	132	17	2
35			1	7	53	148	484	669	273	43	10
12		1		1	12	48	176	212	98	+	1
1						2	15	0	24	9	+

Number of cultures	Distribution of means of F_2 cultures				Average C.V. of F_2 cultures	Distribution of coefficients of variation of F_2 cultures							
	119 128	129 138	139 148	149 158		3 4	5 6	7 8	9 10	11 12	13 14		
1	1				12.0					1			
2	1		1		8.0		1			1			
9	3	2	4	1	8.2		4	1	3				1
20	4	6	9	1	6.1	2	10	7	1				
35	3	13	15	4	5.9	4	23	7		1			
12		5	5	2	5.9	2	4	6					
			1		5.0		1						

Red Algerian bread (No. 3) × early Baart (No. 34)

In 1914 there were grown 6 plants of pure No. 3, 12 plants of pure No. 34 and 6 plants of (3 × 34) F_1 . These numbers are too small to warrant the calculation of coefficients of variation but the distribution and averages may well be given.

TABLE 38
Heights in centimeters in $(3 \times 35) F_2$, 1916.

Number of cultures	F_2 individuals arranged in accordance with the means of the F_2 cultures										
	69 78	79 88	89 98	99 108	109 118	119 128	129 138	139 148	149 158	159 168	169 178
12	1	1	1	25	108	242	150	46	6		
26		2	1	10	43	213	542	402	45	1	1
34				2	13	61	231	797	390	32	2
8						3	22	105	170	71	11

Number of cultures	Distribution of F ₂ parents							Distribution of coefficients of variation of F ₂ cultures							
	109 118	119 128	129 138	139 148	149 158	159 168	169 178	Average C.V. of F ₂ cultures	3 4	5 6	7 8	9 10	11 12	13 14	
12	1	1	3	4	3			8.1		4	4	1	3		
26			2	6	13	5		6.3	1	14	9	1		1	
34		1	3	9	15	5	1	5.8	7	17	8	2			
8			1	1	4	2		5.8		8					

TABLE 39
Heights in centimeters in $(3 \times 34) F_2$, 1914.

Cultures	Number of plants	Average height	100 109	110 119	120 129	130 139	140 149	150 159	160 169
Pure No. 3....	6	118	2	1	2	1			
$(3 \times 34) F_2$...	6	123		1	1	4			
Pure No. 34...	12	150				1	2	7	2

The F_1 is here seen to be intermediate in height between the parents and with a smaller range of variation than either.

Each of the 6 F_1 plants gave rise to an F_2 culture in 1915. For comparison, one culture of No. 3 and one of No. 34 were available. Table 40 gives first summary of the results.

TABLE 40
Heights in $(3 \times 34) F_2$, 1915.

Culture	Number of cultures	Number of plants	Average height in centimeters	Coefficient of variation of the population	Average C.V. of the cultures	Distribution of C. V.	
						3 4	5 6
Pure No. 3..	1	42	146	4.2	4.2	1	
$(3 \times 34) F_2$.	6	537	150	7.1	5.0	1	5
Pure No. 34.	1	92	137	4.1	4.8		1

As in the last bread wheat cross ($\text{No. } 3 \times 35$) and unlike either of the bread wheat \times macaroni wheat crosses (1×35 and 1×3) the average height of the F_2 is greater than the mean of the parents, in fact greater than either of the parents. As usual the coefficient of variation of the F_2 taken as a population was greater than the average of this constant for the separate cultures and the average coefficient of variation of the hybrid cultures was greater than that of the pure parent cultures.

Table 41 gives the distribution of height in the parental races and the F_2 hybrids of this cross.

TABLE 41
Heights in centimeters in (3×34) F_2 , 1915.

Culture	Distribution of individuals										Distribution of means of cultures		
	80	90	100	110	120	130	140	150	160	170	130	140	150
	89	99	109	119	129	139	149	159	169	179	139	149	159
Pure No. 3.....						3	18	21				1	
(3×34) F_2	1			1	6	26	151	232	111	9		2	4
Pure No. 34.....					2	29	55	6			1		

That we should have 4 hybrid cultures averaging taller than the tall parent is interesting, but may be ascribed to hybrid vigor.

The following table (table 42) gives the distribution of the F_2 population grouped according to the height of the F_1 parents, + being the height of F_1 parent, and O the mean of F_2 individuals arising from such parents:

TABLE 42
Heights in centimeters in (3×34) F_2 , 1915.

Number of cultures	Parental height	80	90	100	110	120	130	140	150	160	170	Average height
		89	99	109	119	129	139	149	159	169	179	
1	110				+			O				
	119				1	6	6	41	27	9		147
1	120					+			O			
	129						4	29	42	12		152
4	130						+		O			
	139	1					16	81	190	99	9	155

There is thus seen to be a slight correlation between the height of the F_1 parents and the height of the F_2 , indicating a possibility of some genetic differences in the F_1 in respect to height. In all further discussion of this cross, as regards height, it will be necessary to segregate the data into groups so as to consider at one time only plants originating from a single F_1 parent. Since nearly all of the F_2 population arose

from one or the other of the original F_1 plants, Nos. 25-1 and 44-2, all F_3 cultures except such as originated from these two will be excluded from this study, and these will be kept separate. The distribution of the F_2 of these two cultures were as follows:

TABLE 43
Heights in centimeters in $(3 \times 34) F_2$, 1915.

Culture	Parental height	Number individuals	Average height	Distribution of individuals								Average C.V.
				120	130	140	150	160	170			
(44-2) F_2 , 1915	120	87	152	1	3	29	42	12				4.5
(25-1) F_2 , 1915	135	90	155			12	42	33	3			4.9

The selections for the F_3 covered the full range of both of these parents. Table 44 gives a summary of the results in F_3 .

TABLE 44
Heights in centimeters in $(3 \times 34) F_3$, 1916.

Culture	Number of cultures	Number of individuals	Average height
Pure No. 3.....	5	243	133
$(3 \times 34) F_3$ (44-2).....	50	2408	133
$(3 \times 34) F_3$ (25-1).....	50	2396	131
Pure No. 34.....	5	243	121

Coefficient of variation

Culture	Population	Average of separate cultures	Distribution of C.V.							
			3	5	7	9	11	13	15	
			4	6	8	10	12	14	16	
Pure No. 3.....	8.0	6.6	2	1	1		1			
$(3 \times 34) F_3$ (44-2).....	9.8	6.5	9	23	10	4	2			2
$(3 \times 34) F_3$ (25-1).....	7.7	5.9	10	26	10	3	1			
Pure No. 34.....	7.4	6.2		3	2					

In 1916, it will be observed that the average height of the F_3 is practically the same as the taller parents. The coefficient of variation of the hybrid population is greater than that of the populations of either parent but the average coefficient of variation of the hybrid cultures taken separately was not significantly below that of the pure cultures.

The distribution of the heights of the individuals of the F_3 population and the parental cultures and also of the means of the separate cultures are given in table 45.

Whereas the ranges of the hybrid populations extend beyond the limits of the parents, this is here not surprising considering the much larger

TABLE 45
Heights in centimeters in $(3 \times 34) F_3$, 1916.

Culture	Distribution of individuals																Distribution of means of cultures					
	40	50	60	70	80	90	100	110	120	130	140	150	160	170	100	110	120	130	140	150		
	49	59	69	79	89	99	109	119	129	139	149	159	169	179	109	119	129	139	149	159		
Pure No. 3.....	1			1			1	10	68	108	48	6						3	2			
(3 × 34)F ₃ Total			5	12	13	29	87	453	1570	1819	1068	292	13	1	1	3	41	50	14	3		
(3 × 34)F ₃ (44-2)			3	8	8	18	50	215	652	769	493	179	13		1	2	15	23	8	1		
(3 × 34)F ₃ (25-1)			2	4	5	9	31	184	770	934	428	29				1	21	24	4			
Pure No. 34.....			1			2	22	85	119	14						1	4					

numbers used. It is interesting, however, to note that 17 hybrid cultures had average heights higher than the highest average for the parental cultures.

Table 46 shows the distribution of the F_3 grouped according to the selected F_2 parents. In table 47 the F_3 is grouped according to the means of the F_3 cultures. Table 46 shows a definite correlation between the height of the selected F_2 parent and the mean of the F_3 classes, but there is a strong regression, especially in the higher groups. The F_2 selections, it may be noted, covered practically the entire range of the F_2 population. The distribution of the parents in the F_3 groups of cultures having equal means, was not greater than the normal distribution of individuals in a pure culture. They could therefore be assumed to be modifications (enviromic) of genetically equivalent individuals.

TABLE 46
Heights in centimeters in $(3 \times 34) F_3$, 1916.

Number of cultures	Arrangement of F ₃ individuals grouped according to F ₂ parents												Distribution of means of F ₃ cultures, 1916						
	60	70	80	90	100	110	120	130	140	150	160	170	100	110	120	130	140	150	
	69	79	89	99	109	119	129	139	149	159	169	179	109	119	129	139	149	159	
(44-2)					O		+												
1	2	2	1	9	15	5	10	1	1				1						
8			1	1	2	10	93	O 171	+	91	14				1	5	2		
16			3	3	4	11	71	218	O 233	+	180	53	4		1	7	4	4	
23	1	2	3	3	11	42	218	O 410	O 281	+	124	9				3	15	4	1
2					3	5	35	O 34	O 17		2	+					2		
(25-1)																			
4		1			5	23	O 86	54	+	19						3	1		
22	1	2	2	5	13	97	382	O 410	129	+	6			1	9	12			
22	1	1	3	4	13	64	279	O 423	254	20	+				9	10	3		
2							23	O 47	26	3		+				1	1		

+, Selected F_2 parent.

O, Mean of F_3 group.

TABLE 46 (continued)
Heights in centimeters in (3 × 34) F₃, 1916.

Number of cultures	Mean of F ₃	Average C. V. per cent	Distribution of coefficients of variation of F ₃ cultures						
			3 4	5 6	7 8	9 10	11 12	13 14	15 16
(44-2)									
1	107	16.0							1
8	125	5.8	3	2	2	1			
16	130	6.3	3	8	2	1	2		
23	137	6.4	3	12	5	2			1
2	132	7.0		1	1				
(25-1)									
4	122	6.3	1	1	2				
22	130	6.3	1	15	3	3			
22	133	5.6	7	9	5		1		
2	136	4.0	1	1					

TABLE 47
Heights in centimeters in (3 × 34) F₃, 1916.

Cultures	Arrangement of F ₃ individuals grouped according to means of F ₃ culture												Distribution of selected F ₂ parents					
	69	79	89	99	109	119	129	139	149	159	169	129	139	149	159	169	179	
	60	70	80	90	100	110	120	130	140	150	160	120	130	140	150	160	170	
(44-2)																		
1	2	2	1	9	15	5	10	1	1				1					
2			1	1	7	47	39	2						1	1			
16			5	6	8	18	129	340	207	34	2			5	7	4		
22	1	1				9	35	257	504	235	25			2	4	14	2	
8						1		6	52	206	130	6			4	4		
1									3	17	22	7				1		
(25-1)																		
1						1	22	23	1							1		
21			1	3	4	23	129	517	296	28					3	9	9	
24	2	3	1	1	7	32	225	629	287	12					1	12	10	
4						1	5	58	113	17						3	1	

TABLE 47 (continued)
Heights in centimeters in (3 × 34) F₂, 1916.

Cultures	Mean of F ₂	Average C. V.	Distribution of coefficients of of variation of F ₂ cultures						
			3 4	5 6	7 8	9 10	11 12	13 14	15 16
(44-2)									
1	107	16.0							1
2	118	6.0		2					
16	123	7.6	2	3	6	2	2		1
22	134	6.0	1	16	3	2			
8	147	4.5	6	1	1				
1	153	5.0		1					
(25-1)									
1	120	4.0	1						
21	126	6.2	2	12	6	1			
24	135	5.8	6	12	3	3			
4	142	5.3	2	1	1				

Summary; height

The number of F₁ plants grown were too small to give significant results except in the case of the 1 × 35 and 3 × 35 crosses. In both of these cases the F₁ averaged taller than the tall parent. In the other two cases the F₁ was intermediate. In the two macaroni—bread wheat crosses (1 × 35 and 1 × 3) the F₂ and F₃ averaged below both parental races. In the two bread wheat crosses (3 × 34 and 3 × 35) the F₂ averaged taller than either parent and the F₃ of the 3 × 35 cross was taller than either parent, but in the 3 × 34 cross the average of the F₃ was 1 cm shorter than the taller parent. The distribution of heights in F₁ did not go significantly beyond the limits of the parental cultures in any case except that of 3 × 35 in which the whole distribution was pushed upward about 24 cm. The range of distribution of the individual heights of the F₂ and F₃ in neither case of the macaroni—bread wheat crosses extended significantly above that of the parents, but in both cases extended markedly below the parental range. On the other hand in the bread wheat crosses the range in both cases extended distinctly above, but not significantly below, the parental ranges in F₂ of both crosses and the F₃ of the 3 × 35 cross, but in the F₃ of the 3 × 34 cross it did not extend significantly either above or below the parental range. The same observations made with reference to the distribution of the individual heights of the F₂ and F₃ of both kinds of crosses also apply with perhaps greater emphasis to the distribution of the means of the F₂ and F₃ cultures taken separately.

Now, referring to the appropriate tables, note that the average height

of F_1 in one of the species crosses (macaroni—bread wheat) was above the tall parent and in the other intermediate between the parents. We must therefore assume that the maximum heterozygosity of these crosses will give plants at least taller than the low parent. In both the F_2 and F_3 of these crosses, however, the average F_2 and F_3 height was below the parent. We are therefore compelled to conclude that recombination and not antagonistic heterozygosis is the cause of the low averages of the F_2 and F_3 . A complete double set of macaroni factors, a complete double set of bread wheat factors, or the combination of one complete set of factors from each species, was able to produce a plant of standard vigor, but a large majority of the recombinations of these factors where a complete set from one of the species was lacking, resulted, through failure of coördination, in the production of plants of reduced vigor.

Now it should be noted that no F_2 plant, tall because it was completely heterozygous, could give rise to an F_3 culture which had a high average height, for the reasons above given. Hence the majority of tall F_3 cultures must have arisen from F_2 plants, tall because they were genetically completely, or nearly completely, like one of the parents. Now this is in harmony with the fact (see tables 22 and 31) that the taller F_3 cultures were markedly less variable than were those with a less average height. Now let us remember that the completely heterozygous F_1 plants of the 1×35 cross were tall plants with wrinkled seeds. If we examine the F_2 plants selected and pick out all of those which were taller than the average of the low parent and which also had wrinkled seed, thus again resembling the F_1 plants we find that the average height of the F_3 cultures arising from these were 110 cm with an average coefficient of variation of 19.5 percent, whereas the average height of the offspring of all of the remaining selected F_2 plants taller than the average of the low parent was 123 cm with an average coefficient of variation of 14.1 percent. Again, if we pick out all of the selected F_2 plants which were taller than the average of the low parent and which also had smooth seeds, thus resembling one or the other of the parents, we find that the average height of the F_3 cultures arising from these was 126 cm with an average coefficient of variation of 12.6 percent.

A similar study in the 1×3 cross gave for the F_1 -like F_2 plants F_3 cultures with an average height of 131 cm and an average coefficient of variation of 12.9 percent, whereas the parent-like F_2 plants gave F_3 cultures with an average height of 143 cm and an average coefficient of variation of 6.6 percent.

While these facts coincide completely with the assumptions above

made, the story does not end here. Returning to the 1×35 cross we found that there were 30 tall F_1 -like F_2 plants and 73 tall parent-like F_2 plants. If now we cast the F_3 cultures arising from these two groups respectively into subgroups arranged according to the average heights of the F_3 cultures and find the average coefficients of variation of each subgroup we may tabulate the results as in table 48.

TABLE 48
Average heights of F_3 cultures in centimeters.

		70	80	90	100	110	120	130	140	150
		79	89	99	109	119	129	139	149	159
30 F_3 cultures from tall F_2 plants having the wrinkled seed (F_1 -like F_2 plants)	Distribution of heights	1	2	3	6	8	5	5		
	Average coefficients of variation	30.0	26.0	23.7	20.5	18.5	16.2	16.0		
73 F_3 cultures from tall F_2 plants having smooth seed (parent-like F_2 plants)	Distribution of heights		1	2	5	12	27	20	5	1
	Average coefficients of variation		23.0	16.0	15.6	13.7	13.8	10.0	9.4	5.0

With these results we must conclude that we have not yet succeeded in separating out genetically equivalent groups and that those F_3 plants which gave rise to tall F_3 cultures are genetically more nearly homozygous or else we must postulate some other cause for the suppression of variability in the taller F_3 cultures. This last analysis in no way interferes with the conclusions already drawn, for it clearly shows that in F_3 subgroups of equal height, those cultures arising from F_1 -like plants were always more variable than those which came from parent-like plants.

Now turning to the bread wheat crosses we note that the average coefficients of variation of the F_2 and F_3 generations were in no case significantly higher than that of the most variable parental culture (see tables 33, 35, 40, 44). If, however, we consult tables 38 and 47 we shall observe a distinct lowering of the variability of the taller cultures. Let us also remember that the F_1 , F_2 and F_3 of the 3×35 cross all averaged taller than the tall parent and note (table 38) that the reduction of the variability of the taller F_3 cultures was uniform, whereas the F_1 of the 3×34 cross was intermediate, the F_2 taller and the F_3 again intermediate, and while the reduction in variability of the F_3 cultures (table 47) was still apparent (with the exception of 1 erratic extreme) there was some indication that the intermediate F_3 classes (F_1 -like) had a tendency to be a little more variable. There appears, therefore, to

be two conflicting forces at work, one (heterozygosis) tending to make the cultures arising from the F_1 -like F_2 plants more variable, and another which tends to suppress variability in the taller cultures.

A means of testing for the presence of a factor suppressing variability, which is independent of heterozygosity, is found in the F_2 cultures which came from supposedly genetically equivalent F_1 plants. In the F_2 , the means and variabilities of the several cultures from any given cross should be the same. Where slight differences occur, they are in all probability environic. Nevertheless if the cultures be grouped according to these slight differences in the F_2 means, and the average coefficients of variation of these groups calculated, if there be a factor suppressing variability in the taller groups it should become apparent, provided there is a sufficient number of F_2 cultures to give valid averages. Such an analysis of the F_2 hybrid cultures for 1915 is given in table 49.

TABLE 49
Correlation between average height and coefficient of variation in F_2 hybrids.

		Total number	Average heights, 1915					
			100 109	110 119	120 129	130 139	140 149	150 159
$(1 \times 35) F_2$	Number of cultures	38		4	30	4		
	Average C. V.			19.2	19.0	18.9		
$(1 \times 3) F_2$	Number of cultures	5	2 28	1 5	1	1		
	Average C. V.		28.5	20.2	10.4	14.5		
$(3 \times 34) F_2$	Number of cultures	6					2	4
	Average C. V.						5.5	4.8
$(3 \times 35) F_2$	Number of cultures	18				1	9	8
	Average C. V.					7.0	5.9	6.0

The differences, while not large, are as uniform as could be expected from such small numbers and indicate the presence of a suppression factor of some sort which slightly reduces the variability of the taller cultures.

The presence of this suppression factor for variability in the taller cultures is even more strikingly shown in the pure races. Grouping the cultures according to their means (without regard to year in which they are grown) and calculating the average coefficient of variability for each group we have the result shown in table 50.

Having now shown that there is a factor which, independent of heterozygosity, may suppress the variability of the taller cultures, we may conclude as follows:

(1) Some factor for suppressing variability has been able to com-

TABLE 50
Correlation between average height and coefficient of variation in pure races.

		Total number	Average height				
			110 119	120 129	130 139	140 149	150 159
Pure No. 1	Number of cultures Average C. V.	16		2 7.5	4 7.5	6 6.5	4 5.5
Pure No. 35	Number of cultures Average C. V.	8	3 6.7	3 6.7	1 6.4	1 3.9	
Pure No. 3	Number of cultures Average C. V.	6		2 7.5	3 5.6	1 4.2	
Pure No. 34	Number of cultures Average C. V.	6	1 6.9	4 6.1	1 4.8		

pletely mask the effect of heterozygosity in a cross where the F_2 and F_3 cultures averaged taller than the tall parent (3×34).

(2) This same factor has largely suppressed, but not entirely masked, the variability due to heterozygosity in a cross where the F_2 and F_3 cultures were approximately as tall as the taller parent (3×35).

(3) The factor for the suppression of variability in tall cultures is apparent in crosses where the averages of the F_2 and F_3 cultures are below those of the low parent, but was in no case able to obliterate the effect of heterozygosity (see 1×35 and 1×3).

The question as to the nature of this suppression factor will be reserved for future discussion. The fact that the average variability of the F_2 and F_3 cultures was not significantly higher than that of the pure-line parents in the bread wheat crosses might be cited as showing that a blending inheritance has occurred with the production of a single new race no more variable than the most variable of the parental races, were it not for the fact that tables 37 and 46 show a definite positive correlation between the height of the F_2 parents and the means of the F_3 cultures derived therefrom. A distinct segregation occurred in the formation of the gametes of the F_1 plants whereby the F_2 plants were different genetically and exhibited these differences in the means of their offspring, thus giving rise, not to one race, but to a number of distinct races. The theoretically expected greater variability of the F_2 and F_3 cultures are simply here suppressed, but in the macaroni—bread wheat crosses where this suppression factor was ineffective in masking the variability due to heterozygosis the variability of the F_2 and F_3 cultures in all cases averaged markedly above that of the pure-line parents.

In the F_3 of all crosses, cultures were secured having the parental

types both as regards average height and variability. In the bread wheat crosses the average variability of the F_3 cultures was slightly larger than that of the F_2 cultures in both cases. This is in accordance with the circumstance that in both, the average height of the F_2 cultures was markedly greater than that of the F_3 cultures and thus called into more active effect the variability-suppressing factor already shown to influence the taller cultures. In the macaroni—bread wheat crosses, on the other hand, the average height of the F_2 was greater than that of the F_3 in one case and less in the other, but still the average variability of the F_2 cultures was markedly above that of the F_3 cultures in both cases. This is in harmony with the fact pointed out above that the variability-suppressing factor visible in all of the crosses was not sufficient to mask the influence of heterozygosity in macaroni—bread wheat hybrids.

Finally we may conclude that all of the facts observed in the study of the inheritance of height in the wheat crosses here considered are in harmony with the hypothesis of the segregation of a number of simple Mendelian unit characters and that there is present some factor (as yet unknown) which suppresses variability in the taller cultures of both pure lines and hybrids and that this factor is sometimes able to completely mask the variability which would normally be produced by heterozygosity.

WIDTH OF LEAF

In the following study of the inheritance of width of leaf in wheat hybrids, all measurements are given in millimeters. Averages are therefore given to the nearest millimeter.

Macaroni (No. 1) × Sonora (No. 35)

No pure No. 35 was available for comparison in 1914. The data with reference to the pure No. 1 and the F_1 hybrid plants are given in table 51.

TABLE 51
Width of leaf in millimeters (1 × 35) F_1 , 1914.

	Number of plants	Distribution of individuals																Aver- age	Coefficient of variation
		13	14	15	16	17	18	19	20	21	22	23	24	25	26	27			
Pure No. 1	151	2	1	1	3	11	11	19	25	24	32	5	10	3	3	1	20	13	
(1 × 35) F ₁	39					2	1		4	4	8	9	9	1	1		22	9	

We will here pause only to notice that both the range and variability of the pure No. 1 were greater than for the hybrid. The average leaf

width for the hybrid was greater than for the pure No. 1, but since the No. 1 is here the more narrow-leaved parent we have as yet no indication as to whether or not we are dealing with imperfect dominance or hybrid vigor.

In 1915 there were available for comparison 4 cultures of No. 35, 9 cultures of pure No. 1 and 37 cultures of the $(1 \times 35) F_2$. A summary of these data is presented in table 52.

TABLE 52
Width of leaf in $(1 \times 35) F_2$, 1915.

	Number of head rows	Total number of plants	Average width of leaf	Coefficient of variation of the population	Average C. V. of cultures
Pure No. 1....	9	651	17	13.0	10.3
$(1 \times 35) F_2$...	37	2537	15	30.2	29.3
Pure No. 35...	4	169	20	13.5	13.0

Distribution of coefficients of variation

	7 8	9 10	11 12	13 14	15 16	17 18	19 20	21 22	23 24	25 26	27 28	29 30	31 32	33 34	35 36
Pure No. 1.....	1	5	1	1	1										
$(1 \times 35) F_2$									2	6	6	9	8	5	1
Pure No. 35	1			1	2										

The average of the hybrids is below that of either parent. The standard deviations of the populations are greater than the averages of the standard deviations of the separate cultures making them up, and the variability of the hybrids is much greater than that of the pure cultures. All hybrid cultures were more variable than the most variable pure culture.

Table 53 gives the distribution of the several populations and the distribution of the means of the cultures.

Studying these distributions we note that there were 16 hybrid plants having leaves wider than the widest individual of the widest-leaved parent, but there was no hybrid culture averaging as wide as the most narrow average for Sonora, the wider-leaved parent. On the other hand more than half of the hybrid cultures averaged lower than the lowest average of any macaroni head-row and there were 121 hybrid plants having more-narrow leaves than the narrowest-leaved individual of the macaroni parent.

TABLE 53

Width of leaf in millimeters in (1 × 35) F₂, 1915.

	Distribution of widths in individuals																				Distribution of means of cultures												
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Pure No. 1																																	
(1 × 35) F ₂	3	11	25	46	36	63	93	119	85	198	190	193	260	229	174	190	146	171	103	69	40	39	29	8	9	4	2	1					
Pure No. 35							1			1	1	4	2	6	16	13	19	32	25	24	13	6	4	2									

TABLE 54

Width of leaf in millimeters (1 × 35) F₂, 1915.

Width of parent in 1914	Number of cultures	Average width in 1915	Distribution of leaf widths in individuals																												
			3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29		
17	1	17	1	1					1	2			3	2	4	+	3	3	3	6	2	4		1	1						
18	1	16	2			2			1	1	3	6	6	8	3	11	2	+	4	5	2	2	3	2	1	1					
20	4	15	3	1	3	3	8	7	7	8	19	19	19	27	24	20	23	16	12	+	8	3	4	5	2						
21	4	15	1	1	8	5	8	8	13	8	16	21	15	31	17	21	15	10	24	11	6	4	3	2	5	3	1	1			
22	8	15	4	6	9	6	15	27	32	22	55	43	49	68	54	36	41	29	41	16	21	9	9	2	1	1	1				
23	8	15	4	8	12	13	12	18	27	19	44	46	49	58	54	33	48	31	37	30	13	10	9	9	2	2					
24	9	16	1	2	4	11	6	17	28	23	18	48	45	42	55	46	47	44	40	29	16	9	10	12	3	1					
25	1	15					1	1	3	7	3	4	5	3	8	7	4	5	3	6	3	2									
26	1	15	2	1	1	1	2	5	4	6	4	6	9	5	9	4	5	3	2	1	2	1									

Referring to table 51 it will be observed that there was considerable variation in the width of leaf of the F_1 plants. Table 54 groups the 1915 F_2 plants in accordance with the leaf width of their F_1 parents in 1914.

A glance at this table is sufficient to show that there is no correlation whatever between the parental leaf width in 1914 and the average leaf width of the offspring in 1915. We may therefore conclude that all of the variation observed in the F_1 plants was nutritional and that they were all equivalent genetically so far as the factors governing width of leaf were concerned.

From these F_2 hybrids 230 selections were made which gave rise to a like number of F_3 hybrid cultures in 1916. For comparison with these there were available seven head-rows of No. 1 and five head-rows of No. 35. The selected F_2 plants used as parents ranged in width of leaf from 10 to 35 mm. The very wide-leaved individual was very striking in appearance and was nearly sterile. Table 55 gives a first summary of the results in 1916.

TABLE 55
Width of leaf in millimeters in (1×35) F_3 , 1916.

Class	Number of cultures	Number of individuals	Average width of leaf	Coefficient of variation in the population	Average coefficient of variation of separate cultures
Pure No. 1....	7	344	16	12.0	10.1
(1×35) F_3	230	10123	15	24.9	20.9
Pure No. 35....	5	246	17	15.2	14.0

Distribution of coefficients of variation

Class	9 10	11 12	13 14	15 16	17 18	19 20	21 22	23 24	25 26	27 28	29 30	31 32	33 34	35 36	37 38	39 40	41 42	43 44	45 46	47 48	49 50	51 52	53 54
Pure No. 1.....	5	1	1																				
(1×35) F_3	1	3	24	35	31	42	29	11	17	5	11	5	7	1	3	3	1						1
Pure No. 35.....			3	2																			

The average for the hybrids is less than either of the parents; in every case the coefficient of variation of the population is greater than the average for the pure cultures of the same class and the coefficient of variation for the hybrids is greater than for either parent. The coefficient of variation both for population and average of cultures among the hybrids was lower in 1916 than in 1915. This was also true of the pure

TABLE 56
Width of leaf in millimeters in (1 X 35) F₃, 1916.

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Pure No. 1...											14	24	47	53	78	57	40	21	9		1									
(1 X 35) F ₃ ...	4	27	41	64	53	151	161	255	467	642	1076	904	1268	1074	1151	755	751	383	370	217	106	75	64	15	12	4	2		1	1
Pure No. 35...									1		4	10	24	35	48	27	30	18	21	10	16	1	1							

Distribution of means of cultures

Pure No. 1...											1	1	4	1																
(1 X 35) F ₃ ...							2		2	15	22	30	45	48	27	18	13	5	1	2										
Pure No. 35...															2	1	2													

cultures, and therefore may be in part environic. One thing, however, remains to indicate progressive increase in homozygosity among the hybrids. This is the much greater difference in the coefficient of variation of population and average of cultures, which was apparent in 1916.

Table 56 shows the distribution of the populations of pure cultures and hybrids of this cross in 1916.

The hybrid population shows a distribution far beyond both extremes of the parents. This is also true of the means of cultures. Part of this greater distribution is of course due to the normal extension of the curve from the much larger number of hybrids grown. That the curve of variation is more flat, however, is shown by differences in the shapes of the curves of variation which are rendered comparable by reducing each group class to a percentage of the total number in the population and disregarding all percentages less than one-half of one percent and expressing all percentages to the nearest integer (see table 57).

TABLE 57
Width of leaf in millimeters in (1×3) F_3 , 1916.

	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Pure No. 1.....								4	7	14	15	23	17	12	6	3					
(1×35) F_3	1	1	1	2	2	4	6	11	9	12	11	11	8	7	4	4	2	2	1	1	
Pure No. 35.....								2	4	10	14	20	11	12	7	9	4	7			

When reduced to equal areas the polygon of the F_3 hybrid distribution is thus seen to be limited by a curve much more flat and with more extended limits than either of the parent races. This indicates that the extension of the range of variations of the F_3 hybrids over the parental races is genetic. This is further shown in table 58 where F_3 cultures are thrown into groups or populations in accordance with the leaf width of the selected F_2 parental plants.

Though somewhat erratic at the extremes, these results show a very definite genetic segregation of leaf width in the F_2 as exhibited by the means of their offspring. The distribution of the means of the cultures in each of these groups is shown in table 59.

Width of leaf in millimeters in (1 × 35) F₂, 1916 of the F₁ parents.

Number of cultures	Leaf width of parent	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35		
3	10		3	2	3	2	8	8	10	18	20																										
2	11						1		3	10	4																										
4	12		4	3	3		7	3	16	14	23																										
14	13	1	9	10	14	10	15	13	32	44	79																										
10	14		1	1	2	2	3	6	11	30	51																										
23	15		1	5	3	4	16	24	34	79	87																										
19	16	1	1	3	6	4	14	14	33	36	51																										
24	17	1		7	11	10	24	19	31	53	92																										
28	18		8	2	6	7	17	24	16	57	81	1																									
12	19			1	4	5	8	5	12	20	23																										
32	20		2	1	2	3	10	12	24	36	54																										
22	21			2	1	2	15	10	11	28	33																										
13	22	1	3	3	3	1	4	4	9	14	15	1																									
4	23							2	1	5	2																										
8	24		2	1	4	2	5	15	3	13	10																										
7	25		3		2	1	4	2	5	6	6																										
1	26										1																										
1	27								2	2	7																										
2	28								2	1	1																										
1	35										2																										

+, leaf width of parent; O, mean of F₂ groups.

TABLE 59

Width of leaf in millimeters in (1 × 35) F₂, 1916. Distribution of means of F₂ cultures grouped according to the leaf width of the F₂ parents.

Number of cultures	Parental leaf width in 1915	Mean of group in 1916	8	9	10	11	12	13	14	15	16	17	18	19	20	21
3	10	11	1		1			1								
2	11	13						2								
4	12	12				2	1	1								
14	13	12	1		1	1	6	2	2	1						
10	14	13				1	2	4	3							
23	15	14				3	3	3	6	4	2	1		1		
19	16	14				2	2	3	2	5	4	1				
24	17	14				4	1	5	4	7	2	1				
28	18	14					3	3	14	3	1	3	1			
12	19	14					2		4	3	3					
32	20	15				1	2	3	2	9	7	4	3			1
22	21	16						1	5	5	3	5	3			1
13	22	16				1		1		4	2	1	3			1
4	23	17							1		1		2			
8	24	15						1	1	4		1	1			
7	25	17								2	2	1		2		
1	26	20													1	
1	27	14						1								
2	28	18								1				1		
1	35	19												1		

This table exhibits even more plainly than the preceding the correlation between the parental leaf width and the mean leaf width of the offspring.

In order to determine whether the offspring of narrow-, medium-, and wide-leaved F₂ mother plants exhibited any definite difference in their variability table 60 was constructed.

There is shown here an irregular but still evident diminution of variability among the offspring of the wider-leaved parents.

It may be suggested, moreover, that since width of leaf is highly influenced by the environment and there is therefore a strong regression of the mean of the offspring of extreme variants toward the general mean of the population, we may get a better idea of the segregation of leaf-width factors, by grouping the F₂ cultures according to their own means and then calculating the variability of these groups and observing the distribution of the parents which gave rise to them. We thus measure backward, determining the range of environic modification of individuals which are able to give rise to genetically equivalent progenies.

TABLE 60
Width of leaf in millimeters in $(1 \times 35) F_3$, 1916. Distribution of coefficients of variation of F_3 cultures grouped according to the leaf width of the F_2 parents.

Number of cultures	Parental leaf width	Average C. V. for group
3	10	26.0
2	11	23.0
4	12	24.0
14	13	25.6
10	14	18.5
23	15	20.3
19	16	20.6
24	17	22.0
28	18	21.5
12	19	20.1
32	20	18.3
22	21	19.9
13	22	21.5
4	23	17.8
8	24	22.9
7	25	22.1
1	26	15.0
1	27	22.0
2	28	18.5
1	35	20.0

TABLE 61

Width of leaf in millimeters in (1×35) F_3 , 1916. Distribution of F_3 individuals grouped according to the means of the F_2 cultures. O = mean of group.

Number of cultures	Mean width	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
		1	7	4	4	2	9	6	5	3	3	2	2	1				2		1												
2	8																															
2	10		1	6	6	2	2	3	7	15	O	20	15	3	5	1	4	1														
15	11	3	7	9	13	3	31	36	55	85	95	123	44	50	43	14	8	3	1	1												
22	12		6	5	9	8	25	28	73	83	158	165	120	115	75	48	25	15	4	4	3											
30	13	8	2	7	10	21	33	39	98	114	240	152	182	129	121	34	38	16	13	6	5	1										
45	14	3	6	10	13	34	30	38	94	130	256	248	327	254	232	135	110	44	31	16	8	4	3					1				
48	15	4	4	12	12	21	17	21	53	83	166	194	197	277	O	329	185	177	87	64	42	27	9	5	2	1					1	
27	16	1	5	3	3	5	12	11	18	21	59	72	126	155	213	168	160	73	61	37	27	8	2	1								
18	17						2	1	2	12	16	34	36	71	70	120	110	134	52	77	40	36	11	5	2	1						
12	18						1	2	2	4	6	6	11	28	51	49	50	76	60	64	37	32	15	9	4	4	1	1			1	
6	19							2	2	1	4	6	10	5	14	20	27	30	28	35	20	37	10	19	2	3	2					
1	20								1				1	1	1	1	2	2	6	9	3	12	4	6	1							
2	21										1		1		4		8	5	9	10	11	11	13	12	3	3	1					

TABLE 62
Width of leaf in millimeters in (1×35) F_2 , 1916. Distribution of F_2 parents grouped according to the means of the F_3 cultures.

Number of cultures	Mean of F_2 cultures	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
2	8			1			1																						
2	10			1																									
15	11					2	1	1	3	2	4																		
22	12					1	6	2	3	2	1	3	2																
30	13						2	4	3	3	5	3																	
45	14					1	2	3	6	2	4	14	4	2	5			1			1								
48	15						2	3					3	9	5	4													
27	16							1	4	5	7	3	3	7	3	2	1		4	2		1							
18	17								2	4	2	1	3																
12	18								1	1	1	3							1										
6	19											1																	
1	20																												
2	21																												

TABLE 63
Width of leaf in millimeters in (1×35) F_3 , 1916. Distribution of coefficients of variation of F_3 cultures grouped according to the means of the F_3 cultures.

Number of cultures	Means of F_3 cultures	Average C.V. of F_3	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39	41	43	45	47	49	51	53
2	8	43.5																							
2	10	30.5																							
15	11	25.6		1			2	2		2	2			1	2		1		1						
22	12	22.9																							
30	13	22.2		1		1	5	2	3	2	4	2	2												
45	14	21.4					4	5	4	2	2		3					1	1						
48	15	20.8			3	6	3	10	10	2	4	1	2	1	2										
27	16	18.4			10	6	8	8	4	1	3	1	3	2		1	1								1
18	17	17.4			4	6	4	8			2	1													
12	18	17.4		1	2	5	2	4	3	1															
6	19	18.6			2	4	1	3	2																
1	20	15.0			1	1	1	1	2																
2	21	14.5			1	1																			

[illegible]

The average leaf width of the hybrids is below that of either parent. The coefficient of variation of the populations are greater than the averages of the separate cultures and the variation of the hybrids is greater than that of the most variable pure culture.

Table 66 gives the distribution of the individuals of the several populations and the distribution of the means of the separate cultures.

TABLE 66
Width of leaf in millimeters in $(1 \times 3) F_2$, 1915.

Class	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Pure No. 1.....					2	1	2	1	2	6	28	62	75	117	130	107	78	26	11	3			
$(1 \times 3) F_2$	2	1	3	7	15	13	20	30	30	33	37	49	44	32	30	17	19	9	6	4	1	3	1
Pure No. 3.....										1	1	2	7	11	8	9	1	1	1				

Distribution of means of cultures.

Class	13	14	15	16	17	18	19
Pure No. 1				4	1	2	2
$(1 \times 3) F_2$	3		2	1			
Pure No. 3				1			

We first note that, notwithstanding the fact that there were nearly 200 more individuals in the population of No. 1 than in the hybrid population, still the range of leaf width among the hybrids extended markedly beyond the range of pure No. 1 in both directions, and this in spite of the fact that no single hybrid culture averaged greater than the narrowest-leaved culture of pure No. 1.

Now analyzing the relation of the F_2 hybrid cultures to their (F_1) parents we find that there is a possibility that there were some differences in the genetic constitution of the F_1 plants inasmuch as the narrow-leaved parents produced offspring with a lower average leaf width than did the wider-leaved parents. This is shown in table 67.

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TABLE 67
Width of leaf in millimeters in ($I \times 3$) F_2 , 1915.

Plant No. 1914	Width of leaf, 1914	Average width of leaf of offspring, 1915	Distribution of leaf width in offspring, 1915																							
			4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
33-1	13	13	1			3	5	4	3	9	5	+	0	3	7	8	4	3	1							
33-2	14	13				1	1	1	4	1	1	2	4	1	2			1	1							
49-7	16	13				1	1	3	4	3	4	7	3	9	9	3	1	3		1						1
32-1	20	15						4	2	2	5	4	4	7	9	7	8	7	3	+						
52-2 (2nd head-row)	23	15	1	1	2	1	1	2	2	6	8	8	7	15	8	9	7	5	8	5	1	+				
52-5 (1st head-row)	23	16				1	1	1	6	5	5	10	7	8	10	9	7	7	4	3	+					1

Now grouping these cultures according to their mean in 1915, table 68 gives the average and distribution of the coefficients of variation of these groups.

TABLE 68
Width of leaf in millimeters in (1 × 3) F₂, 1915.

Number of cultures	Average leaf width of culture in 1915	Average coefficient of variation	Distribution of C.V. of cultures					
			21	23	25	27	29	
			22	24	26	28	30	
3	13	26.7			2			1
2	15	26.5			1	1		
1	16	22.0	1					

The coefficients of variation here show a strong decline in variability in the wider-leaved cultures.

In 1916 there were available for comparison 7 cultures of pure No. 1, 5 of pure No. 3 and 57 cultures of the F₃ hybrid 1 × 3. Table 69 summarizes the results obtained.

TABLE 69
Width of leaf in millimeters in (1 × 3) F₃, 1916.

Class	Number of cultures	Total number of plants	Average leaf width	Coefficient of variation of the population	Average C. V. of separate cultures
Pure No. 1	7	344	16	12.0	10.1
(1 × 3) F ₃ (33-1)	9	406	12	21.3	18.1
(1 × 3) F ₃ (49-7)	8	365	13	24.1	21.4
(1 × 3) F ₃ (32-1)	40	1763	13	26.5	20.9
(1 × 3) F ₃ (Total)	57	2534	13	25.3	20.5
Pure No. 3	5	243	14	12.2	11.4

TABLE 70

Width of leaf in millimeters in (1 × 3) F₂, 1916.

Class	Distribution of individuals																										
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
Pure No. 1.....											14	24	47	53	78	57	40	21	9		1						
(1 × 3) F ₂ (33-1) ..		3		3	4	12	9	22	48	71	68	69	41	21	21	7	2	2	2								
(1 × 3) F ₂ (49-7) .		4	4		2	6	6	17	23	66	38	47	38	37	30	27	9	7	4		1						
(1 × 3) F ₂ (32-1) ..	2	7	5	14	18	66	46	83	77	187	176	205	178	235	161	129	80	39	35	12	7					1	
(1 × 3) F ₂ (total)	2	14	9	17	24	84	61	122	148	324	282	321	257	293	212	163	91	48	41	12	8					1	
Pure No. 3.....								3	3	7	23	30	66	54	44	7	4	2									

Distribution of means

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Pure No. 1.....																	
(1 × 3) F ₂ (33-1) ..									1	3	3		1	1	1	4	1
(1 × 3) F ₂ (49-7) ..									1		2	3	1	1	1		
(1 × 3) F ₂ (32-1) ..								1	2	5	8	7	8	2	3	2	2
(1 × 3) F ₂ (total) .								1	4	8	13	10	10	3	4	2	2
Pure No. 3.....												1	3	1			

Distribution of coefficients of variation

	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37
	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38
Pure No. 1.....	5	1	1												
(1 × 3) F ₂ (33-1) ..		1	2	1		1	1	2				1			
(1 × 3) F ₂ (49-7) ..					1	3	1	2							1
(1 × 3) F ₂ (32-1) ..	2	9	12	6	3	2	2		2			1	1	1	1
(1 × 3) F ₂ (total) .	3	11	13	7	7	3	1	4	2			1	2	1	2
Pure No. 3.....	3	1		1											

A study of tables 69 and 70 will show that it is not worth while to treat separately the 1×3 hybrids originating from the different original pollinations, since their means and distributions were practically equal. They will therefore be treated together hereafter.

In table 69 we observed that the average leaf width of the hybrids was below both the parents. The coefficient of variation was, however, as usual, markedly higher for the hybrids. From table 70 we note that the hybrid range in leaf width extends from a single case markedly above both parents to plants with almost filiform leaves. The different hybrid groups show practically the same behavior. Whereas 3 hybrid cultures showed as little variability (coefficients of variation) as the least variable parental culture, more than half were more variable than the most variable parental culture.

There were 8 hybrid cultures whose mean leaf widths were as great or greater than the mean for the wider-leaved parent. It is, moreover, interesting to note that from the hybrids of parents differing, on the average, only 2 mm in leaf width, there have segregated out races whose average leaf width differs by 9 mm. The fact that a large part of the differences in leaf width observed in the F_2 generation were genetic, is shown in table 70 which exhibits the F_3 cultures grouped according to their parental leaf widths.

There is a distinct correlation between parental leaf width and the mean of the offspring. Whereas the means show a marked range of distribution in each of the parental groups, this range is never wider

TABLE 71
Width of leaf in millimeters in $(1 \times 3) F_2$, 1916.

F_2 individual plants grouped according to the heights of F_2 parents

Number of cultures	Leaf width of parent	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
2	8	1	3		4	7	9	4	11	4	8	5	5	2			1		1								
1	9						6	3	6	5	4	4	4	2	1												
1	11									4	17	10	7	4		2		1									
4	13					3	3	5	10	7	38	31	34	12	20	7	4	2	1	2		1					
5	14			1	2	3	4	5	13	7	36	36	33	33	23	20	9	5		1							
11	15		6	6	2	7	22	14	23	35	74	66	71	44	50	32	26	8	4								
12	16	1	1	1	4		18	14	35	35	72	55	59	70	76	54	39	12	3	3		1					
6	17		1			1	8	3	6	14	29	28	43	32	46	18	15	10		1	1						
6	18		2		1	1	8	8	13	25	38	36	34	29	25	19	17	4	5	3							
2	19										1	11	9	24	19	15	7	5	3	1							
3	20				4	2	4	5	3	5	4	6	6	9	6	6	9	15	17	14	5	4					1
2	22		1	1			1		1	5	2	2	3	6	10	15	12	14	9	6	4	1					
1	23						1		1	2	2	2	8	3	5	7	4	5	1	4	1						
1	25												3		6	12	12	8	2	4	1				+		

Means of cultures, 1916

Number of cultures	Mean of group 1916	9	10	11	12	13	14	15	16	17	18
2	9	1	1								
1	11			1							
1	12				1						
4	12			1	2		1				
5	13				2	2	1				
11	12		1	2	3	4	1				
12	13		1	2	1	3	3	1	1		
6	13				3		2	1			
6	13			2	1	1	1	1			
2	16								2		
3	15			1						1	1
2	16										1
1	16								1		
1	17									1	

+ = leaf width of parent

○ = average leaf width of offspring

than the fluctuations of the individuals of a pure line. The coefficients of variation (see table 72) show a distinct though irregular decline toward the wider-leaved parental groups.

TABLE 72

Width of leaf in millimeters in $(1 \times 3) F_3$, 1916. Coefficients of variation of F_3 cultures grouped according to the leaf width of the F_2 parents.

Number of cultures	Parental leaf width	9 10	11 12	13 14	15 16	17 18	19 20	21 22	23 24	25 26	27 28	29 30	31 32	33 34	35 36	37 38	Average C. V. of group
2	8													I		I	35.5
1	9								I								24.0
1	11			I													14.0
4	13				2	I					I						18.8
5	14				I	I	I	I		I							20.0
11	15	I			I	I	2		2	3			I			I	23.5
12	16	I	I	I	I	I	2	I	3			I					19.0
6	17			I	I	I	I			I	I						19.8
6	18	I	I			I	I					I	I				19.7
2	19		I	I													12.0
3	20	I								I					I		23.3
2	22	I									I						18.5
1	23						I										19.0
1	25	I															10.0

This study of variation is made much more distinct by regrouping the F_3 cultures according to their own means in 1916, as in table 73.

TABLE 73

Width of leaf in millimeters in $(1 \times 3) F_3$, 1916. Distribution of F_3 individuals grouped according to the means of the F_3 cultures.

Number of cultures	Mean of culture	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	9	1	3	3	4	2	3	10	2	7	5	3															
4	10			1	8	15	9	19	27	40	13	11	4	2		I		2									
8	11		4	8	4	30	24	39	42	51	37	34	34	17	7	4	I	2			I						
13	12		4	5	1	6	18	12	29	45	130	100	93	40	46	20	12	6	3	I	I	I					
10	13	I	1	2	1	I	12	7	19	20	61	63	59	57	53	49	29	10	4	2		I					
10	14		2	2	3	I	6	4	6	10	41	38	71	63	84	57	48	18	3	5	2				I		
3	15								2	I	5	25	28	33	12	19	6	I	I								
4	16					I		I	2	2	3	20	20	46	35	27	15	7	8	I	I						
2	17						2		3		2	4	3	7	13	15	12	11	6	3	2						1
2	18							I					4	6	13	9	23	15	18	4	2						

TABLE 74
Width of leaf in millimeters in (1 × 3) F₂, 1916.

Number of cultures	Mean of F ₂ cultures	Distribution of F ₂ parents																							
		8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25						
1	9	1																							
4	10	1					1		1	1															
8	11		1						2	2		2		1											
13	12				1		2	2	3	1	3	1													
10	13							2	4	3		1													
10	14						1	1	1	3	2	1				1									
3	15									1	1	1													
4	16																								
2	17												2				1								
2	18													1					1						
2	18													1		1									

Number of cultures	Mean of F ₂ cultures	Coefficients of variation of F ₂ cultures grouped according to the means of the F ₂ cultures																Average C V. of group
		9	11	13	15	17	19	21	23	25	27	29	31	33	35	37		
		10	12	14	16	18	20	22	24	26	28	30	32	34	36	38		
1	9												1			33.0		
4	10					1	1		1						1	29.8		
8	11						1	1	2	1		1	1		1	26.1		
13	12	1	1	2	1		2			3	2				1	21.1		
10	13				1	3	1		3	1		1				21.2		
10	14				3	2	2	1			1		1			19.9		
3	15	1	1	1												11.7		
4	16	1	1	1			1									13.0		
2	17	1								1						17.5		
2	18	2														10.0		

A study of table 74 shows very plainly that there is a distinct and marked segregation of leaf-width factors in the F₂ which gives rise to F₃ cultures whose averages reach or exceed the parental means in both directions. As measured by the coefficient of variation, the variability of the hybrid cultures clearly decreased as the average leaf width increased. Does this mean that the wide-leaved cultures are more nearly homozygous (on the average) than the narrow-leaved segregates? If this were true it would follow that the factors tending to increase leaf width are recessive and that the genetically narrow-leaved plants were so on account of dominant inhibitors. This idea is, however, not supported by the fact that the leaf width of the F₁ plants (see tables 51 and 54) which had the maximum of heterozygosity, has leaf widths

averaging as high or higher than either parent. If leaf-width inhibiting factors are dominant the maximum narrowness should occur in the F_1 plants. If on the other hand these factors exhibited imperfect dominance one would expect the medium races to have a higher variability than those approaching the extremes. Such, however, is not the case. We must therefore seek elsewhere for the explanation of this decrease in variability as the average leaf width of the cultures increases.

Inheritance of leaf width in bread wheat crosses, Sonora (No. 35) \times red Algerian bread wheat (No. 3)

As previously mentioned no pure No. 35 was available for comparison with the F_1 generation in 1914. A comparison of the leaf width of pure No. 3 with the (3×35) F_1 hybrid plants is given in table 75.

TABLE 75
Width of leaf in millimeters in (3×35) F_1 , 1914.

	Number of plants	18	19	20	21	22	23	24	25	26	Average leaf width
Pure No. 3	3	1		1			1				20
(3×35) F_1	18			4	2	4	3	4		1	22

While the numbers here given are too small to form the basis of definite conclusions, they at least indicate that the F_1 hybrids have leaves as wide as, or wider than, the parents.

These 18 F_1 plants gave rise to 18 plant rows of F_2 hybrids in 1915 and there were available for comparison with them 1 pure culture of No. 3, and 4 pure cultures of No. 35. The results may be summarized as in table 76.

TABLE 76
Width of leaf in millimeters in (3×35) F_2 , 1915

Class	Number of cultures	Number of individuals	Average leaf width	Coefficient of variation of the population	Average C. V. of cultures	Distribution of C.V.					
						7 8	9 10	11 12	13 14	15 16	
Pure No. 3	1	42	16	11.2	11.2			1			
(3×35) F_2	18	1620	18	13.9	13.4		1	2	12	2	
Pure No. 35	4	169	20	13.6	13.0	1			1	2	

The mean leaf width of the hybrids is intermediate between the parents. The average variability of the hybrids is only slightly above that of the pure cultures.

The distribution of the populations and means for this generation are given in table 77.

TABLE 77
Width of leaf in millimeters in (3 × 35) F₂, 1915.

	Distribution of individuals																										Distribution of means of culture					
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	16	17	18	19	20	21					
Pure No. 3							1	1	2	7	11	8	9	1	1	1						1										
(3 × 35) F ₂	1		3	2	3		7	17	74	113	191	256	259	225	213	127	70	31	22	5				2	14	2						
Pure No. 35			1			1	1	4	2	6	16	13	19	32	25	24	13	6	4	2					1	2	1					

It is interesting here to note that the distribution of the means of the hybrids did not reach the extremes of the parents and that although the number of hybrids was many times that of No. 35, the range of variation of the hybrids toward wide leaves did not exceed that of its broad-leaved parent.

For the F₃ of this cross there were available 5 pure cultures of each of Nos. 3 and 35, and 80 plant rows of (3 × 35) F₃. The hybrid F₂ plants chosen for planting in the fall of 1915 included 11 of the 19 classes through which the population of F₂ was distributed. A first tabulation of the results follows:

TABLE 78
Width of leaf in (3 × 35) F₃, 1916.

Class	Number of cultures	Number of individuals	Average leaf width	Coefficient of variation of the population	Average coefficient of variation	Distribution of C. V.									
						9 10	11 12	13 14	15 16	17 18					
Pure No. 3	5	243	14	12.2	11.4	3	1			1					
(3 × 35) F ₃	80	3852	17	15.5	12.9	6	29	28	11		6				
Pure No. 35	5	246	17	15.2	13.8			3	2						

One is surprised to find here the mean of the F₃ hybrids as high as the broader-leaved parent and the average coefficient of variation of the separate cultures of hybrids lower than that for the Sonora (No. 35).

The distribution of the individuals in the populations of hybrids and pure cultures is shown in table 79.

TABLE 79
Width of leaf in millimeters in (3 × 35) F₃, 1916.

	Distribution of individuals																									Distribution of means of cultures									
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	13	14	15	16	17	18	19								
Pure No. 3																					1	3	1												
(3 × 35) F ₃	3	3	4	1	7	52	97	223	303	583	554	589	392	392	324	207	69	33	14	2		4	13	20	15	20	8								
Pure No. 35				1		4	10	24	35	48	27	30	18	21	10	16	1	1						2	1	2									

It is particularly interesting to note here that there were ten cultures with means higher than the highest mean for the wide-leaved parent. We have here a suggestion that if there be some force limiting variability in the wider-leaved races it would more strongly affect these wide-leaved hybrid cultures and thus aid in reducing the average variability of the group. In this connection it may be remarked that the average coefficient of variability of these ten cultures is 11.4 percent, a figure well below the average coefficient of variability for pure No. 35, which is 13.8 percent.

It is also interesting to note that whereas in the macaroni—bread wheat crosses many cultures were grown, the average leaf widths of which were below that of the narrow-leaved parent, here we have no cultures lower, but there are eight above the wider-leaved parent.

The segregation and recombination of characters by which these markedly different races were isolated is shown in table 82 where the F_3 individuals are grouped according to the mean leaf width of the F_3 cultures.

TABLE 80
Width of leaf in millimeters in (3×35) F_3 , 1916. Population grouped according to the leaf width of the F_2 parents.

Number of cultures	Parental width in 1915	Distribution of F ₃ grouped according to leaf width of F ₂ parents																										Average in 1916	
		6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26							
2	14						3	6	17	22	0	16	5	4	2														14.6
2	15	I				I	4	11	10	14	13	19	12	5	3	I													14.8
9	16		I			2	9	16	56	51	84	76	61	40	19	13	5	3											15.6
14	17	I		I			14	18	44	70	124	124	115	72	47	32	14	2	3										16.1
11	18	I		3	I	I	11	16	30	41	96	65	82	46	61	36	26	11	4										16.6
9	19						2	8	20	24	62	64	65	54	49	38	32	6	2	2	I								17.2
15	20		I			3	6	18	25	59	102	113	104	71	78	69	38	16	7										16.9
7	21							2	9	11	22	29	56	39	62	62	29	9	4										18.1
6	22		I						1	6	8	26	21	49	33	36	43	36	10	10	I								18.4
4	23						2			4		9	17	32	23	32	30	26	11	3	2								18.5
1	24						I	I	I	3	14	10	8	5	3		I	I											16.1

This table shows a regular and nearly uniform correlation between the parental leaf width and the average leaf width of the offspring. The

one exception at the wide extreme came from plant No. 21-5-2-1, a plant which stood at the end of the row and was very likely an extreme variant of about the 18 class (see range of this class in table 80).

TABLE 81
Width of leaf in millimeters in (3 × 35) F₃, 1916.

Number of cultures	Leaf width of F ₂ parents	Distribution of means of F ₃ cultures grouped ac- cording to leaf width of F ₂ parents								Average coefficient of variation of F ₃ cultures	Distribution of coefficients of variation					
											9	11	13	15	17	
		14	15	16	17	18	19	20	10		12	14	16	18		
2	14	I	I						11.5		2					
2	15	I	I						15.5			I		I		
9	16	I	3	4	I				13.3	I	2	3	2	I		
14	17		4	5	4	I			12.5	2	5	5	I	I		
11	18	I	3	I	2	3	I		13.4	I	4	3	I	2		
9	19			3	3	2		I	12.6	I	4	3	I			
15	20		I	6	3	4	I		13.7		4	7	3	I		
7	21				I	4	2		12.0	I	3	3				
6	22				I	2	2	I	13.2		3		3			
4	23					2	2		12.0		2	2				
I	24			I					13.0			I				

There is an indication of some decline in the coefficient of variation in the wider-leaved groups, but it is too much broken up by irregularities to be of any particular significance.

The study of variability of the F₃ is better made, however, by re-grouping the F₃ cultures in accordance with their own means. This is done in table 82.

TABLE 82
Width of leaf in millimeters in (3 × 35) F₃, 1916. Population grouped according to the average leaf width of the F₃ cultures.

Number of cultures	Average leaf width of F ₃ cultures	Distribution of leaf widths of individuals																			
		6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
4	14	1				3	12	19	31	36	40	31	15	3	1	1					
13	15			3	1	1	20	28	83	94	146	100	82	36	20	10	2				
20	16	1	2			2	14	31	59	94	209	170	164	83	70	33	23	8	1		
15	17		1				3	12	27	48	104	126	114	93	88	64	25	10	5		
18	18	1	1		1	3	6	21	27	64	95	150	118	136	129	80	26	7	1		
8	19								3	17	30	57	50	53	72	58	20	10	5		
2	20							1	1	1	3	2	7	9	14	15	19	5	10	8	2

○ = means of F₃ groups.

Comparing tables 78 and 82 we note that, starting with cultures which differed on an average by 3 mm in leaf width, we have obtained cultures whose means differ by 6 mm.

TABLE 83

Width of leaf in millimeters in (3×35) F_3 , 1916. F_2 parents of F_3 cultures grouped according to the means of the F_3 cultures.

Number of cultures	Mean of F ₃ cultures	Distribution of F ₂ parents												Average C. V. of F ₃ cultures	Distribution of C.V. of F ₃ cultures				
		14	15	16	17	18	19	20	21	22	23	24	9 10		11 12	13 14	15 16	17 18	
4	14	1	1	1		1							13.8			2	1		1
13	15	1	1	3	4	3		1					13.4			5	6		2
20	16			4	5	1	3	6				1	13.6	1	5	7	5		2
15	17				1	4	2	3	3	1	1		13.1	2	2	8	3		
18	18					1	3	2	4	4	2	2	12.7	1	9	5	2		1
8	19						1		1	2	2	2	11.2	2	5	1			
2	20							1				1	13.5		1		1		

From table 83 we observe that the range of parents which may give rise to an offspring with a given mean is not greater than that of a pure culture.

When the coefficients of variation are calculated we find an irregular but still quite definite decline toward the wider-leaved cultures as usual (see table 83).

Algerian red bread (No. 3) \times early Baart (No. 34)

This cross will be of special interest for comparison with the other crosses inasmuch as the two parents had practically the same width of leaf. The number of plants grown in 1914 are too small to furnish trustworthy averages but as a matter of record they may be given as follows:

TABLE 84

Width of leaf in millimeters in (3×34) F_1 , 1914.

Class	Number of plants	Average leaf width	Distribution of leaf widths											
			14	15	16	17	18	19	20	21	22	23	24	
Pure No. 3	3	19					I		I					
(3 × 34) F ₁	6	20	I			I			I	3			I	
Pure No. 34	12	21					I	I	4	3	2		I	

TABLE 85

Width of leaf in millimeters in (3 × 34) F₂, 1915.

Class	Leaf width of parent F ₁	Average leaf width F ₂	Distribution of individuals													Number of plants	C. V.		
			10	11	12	13	14	15	16	17	18	19	20	21	22				
Pure No. 3.....	20	16				1	1	1	2	7	11	8	9	1	1	1	42	11	
(3 × 34) F ₂ (44-1).....	14	15				8	4	28	23	9	10	3	3	2			91	12	
(3 × 34) F ₂ (44-2).....	17	16	1			2	2	7	16	19	13	18	7	3			81	11	
(3 × 34) F ₂ (25-1).....	20	18								1	5	6	18	22	16	18	5	91	9
(3 × 34) F ₂ (28-1).....	21	16				1	1	8	18	30	14	18	1				91	9	9
(3 × 34) F ₂ (47-1).....	21	18							1	2	5	16	21	25	13	5	1	89	8
(3 × 34) F ₂ (47-2).....	21	18							1	4	9	8	32	13	13	7	2	89	9
Pure No. 34.....	18	17				1	2	9	18	13	17	18	10	3	1		92	11	11
Averages and totals for (3 × 34) F ₂	19	17	1	11	7	46	68	78	79	114	65	49	7	3			532	9*	

* Coefficient of variation of hybrid population = 12.

TABLE 86

Width of leaf in millimeters in (3 × 34) F₂, 1916.

Class	Number of cultures	Number of individuals	Average width of leaf	C. V. of population	Average C. V. of cultures	Distribution of C. V.													
						7	8	9	10	11	12	13	14	15	16	17	18	19	20
Pure No. 3	5	243	14	12.2	11.4									3	1	1			
(3 × 34) F ₂ (25-1)	50	2387	16	13.3	12.0									1	13	16	13	7	
(3 × 34) F ₂ (44-2)	49	2336	15	12.9	11.8									1	11	21	14	1	1
Pure No. 34	5	243	14	12.6	11.4											5			

These 6 F_1 plants gave rise to 6 plant rows of F_2 hybrids in 1915 and there were available for comparison 1 pure culture of each of Nos. 3 and 34. Since the F_2 cultures differed somewhat in accordance with the leaf width of the F_1 plants, the records will be given in full rather than being summarized as usual (table 85).

Here we have the average of the hybrids less variable than either parent. It should be observed that the one hybrid culture (No. 44-1) which was more variable than either parent had a mean lower than either parent and that the three cultures having means higher than either parent all had coefficients of variation well below either parent. The mean of all of the F_2 was equal to the wider-leaved parent and the total range of the F_2 was practically confined to the limits of the parental range. The means of the F_2 cultures varied on either side of the parental means but in such cases kept their total range inside of the parental range by narrowing their own variability.

In view of these rather marked discrepancies in the means of the F_2 cultures subsequent study is confined to the progenies of but two F_1 plants (44-2 and 25-1) and these are kept separate.

In 1916 there were available for study 5 plant rows of each of the parental cultures, pure No. 3 and pure No. 34, selected from these strains of the previous season and for the hybrids 50 selections from the F_2 of 25-1 and 49 selections from the F_2 of 44-2.

Here the means of the hybrids are above the means of either parent but unlike the F_2 the coefficients of variation are slightly above that of the parental cultures. In table 87 we note that some of the hybrid cultures were more and some were less variable than certain of the pure

TABLE 87

Width of leaf in millimeters in (3 × 34) F_2 , 1916. Distribution of the populations and means of cultures of hybrids and parents.

	Distribution of population																			
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
Pure No. 3.....			3	3	7	23	30	66	54	44	7	4	2							
(3 × 34) F ₂ (25-1) ..				7	24	69	119	327	405	555	297	321	109	98	38	14	3		1	
(3 × 34) F ₂ (44-2) ..	2	1	2	15	61	188	249	447	514	451	178	154	48	20	5	1				
Pure No. 34.....			2	6	25	37	34	67	47	16	7	2								
Distribution of means of cultures																				
Pure No. 3.....							1	3	1											
(3 × 34) F ₂ (25-1) ..								2	13	19	13	3								
(3 × 34) F ₂ (44-2) ..							3	13	22	9	2									
Pure No. 34.....						1		4												

cultures. The differences obtained are, however, not large enough to have any especial significance.

In table 87, the most interesting feature is the distribution of the means. Here we have 46, approximately half, of the hybrid cultures with means higher than either of the parents. The same was true in the F_2 cultures (see table 85). As regards height, it will be recalled that the hybrids of this class also averaged as high or higher than the taller parent. The fact that so many races had average leaf widths so strikingly above either parent would suggest recombination with the production of races beyond the extremes of the parent. This, however, is made very doubtful by a study of table 88. There the F_3 cultures are grouped according to the leaf width of the F_2 parents. Moreover, seeds were planted from each of the plants of the F_2 of the populations of the cultures concerned (25-1 and 44-2). If therefore the variations in leaf width of the F_2 plants were partially genetic and partially nutritional (enviromic) the averages in the F_3 groups should show a correlation with their F_2 parents.

We do not seem to have any correlation whatsoever between the leaf width of the parent and offspring. We may therefore conclude that so far as this character is concerned the F_2 plants were all genetically equivalent and that all differences such as did arise were modifications.

A study of the distribution of the means of the F_3 cultures grouped according to their F_2 parents also confirms the conclusions already drawn that the F_2 plants were all equivalent genetically so far as leaf

TABLE 88

Width of leaf in millimeters in (3 × 34) F₃, 1916.

Number of cultures	Parental width F ₂ 1915	Average leaf width F ₃ , 1916	Distribution of individuals in F ₃ cultures																Distribution of means of F ₃ cultures								
			7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	13	14	15	16	17	18
(25-1)																											
1	14	16									2	1	3	+	7	12	8	12	2								1
3	15	16						1	1	2	10	18	22	21	26	26	8	5	1								1
3	16	15							4	3	7	38	31	45	9	9	1	1									3
9	17	16						3	3	19	21	51	62	93	63	54	17	23	8	2	1						1
6	18	16						3	4	3	6	42	45	65	31	36	17	16	9	4							1
10	19	16						6	18	25	76	79	125	48	68	18	16	6	4								1
14	20	16						5	17	38	81	126	145	88	90	35	26	13	4	2							2
4	21	16						1	5	11	18	33	46	24	26	13	9	1									1
(44-2)																											2

I	12	13																									
3	14	15																									
7	15	15																									
11	16	15																									
7	17	15																									
12	18	15																									
5	19	15																									
3	20	15																									

+ = selected F₂ parents. O = means of F₃ cultures.

width was concerned. However, both tables indicate that the strain originating from the original hybrid plant 25-1 had slightly broader leaves than that originating from the original hybrid plant 44-2.

TABLE 89

*Width of leaf in millimeters in (3×34) F_3 , 1916.
Population grouped according to the average leaf width of the F_3 cultures.*

Number of cultures	Average leaf width of	Distribution of individuals																							
		F ₃ cultures	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25				
(25-1)																									
2	14				2	3	9	10	27	20	18	4	3	1	1										
13	15				1	11	30	50	133	132	148	55	53	6	6	1									
19	16				4	7	27	42	101	163	227	134	117	35	28	10	3	1							
13	17					3	12	16	59	74	144	84	122	53	40	13	6				1				
3	18						1	1	7	16	18	20	26	14	23	14	5	2							
(44-2)																									
3	13				5	15	33	26	36	21	4	3	1												
13	14			1	8	24	75	92	156	135	95	25	10	2											
22	15	2	1	1	2	18	73	115	187	239	225	88	75	16	6	2									
9	16				4	14	12	62	104	111	44	58	17	6											
2	17						3	4	6	15	16	18	10	13	7	3	1								

O = means of F_3 groups.

In order better to study the variability of the F_3 generation of this cross, the plants were regrouped according to the means of the F_3 cultures in table 89, and table 90 gives the distribution of the F_2 parents and the coefficients of variation of the F_3 cultures in the same grouping.

The distribution of the F_2 parents in this arrangement appears entirely fortuitous without any correlation whatsoever with the means of the progenies to which they gave rise. These facts therefore form additional evidence that the F_2 plants were all equivalent genetically and that all variations of individuals in the F_2 or of means of cultures in the F_3 were due to non-genetic factors.

We are unable to detect any significant difference in the coefficients of

TABLE 90

Width of leaf in millimeters in (3×34) F_3 , 1916. F_2 parents and coefficient of variation of F_3 cultures grouped according to the means of the F_3 cultures.

Number of cultures	Means of F ₂ cultures	Distribution of F ₂ parents										Average C. V. of group	Distribution of the coefficients of variation															
		12	13	14	15	16	17	18	19	20	21		7	9	11	13	15	17	19	21								
(25-1)																												
2	14							1	1			13.5			1		1											
13	15				1	3	1	1	4	2	1	11.5			5	3	5											
19	16			1	1		5	2		8	1	12.1	1	4	7	4	3											
13	17				1		2		5	3	2	9.4		4	3	3	3											
3	18						2			1		12.3			2	1												

(44-2)																												
3	13	1					1		1			12.0		1		2												
13	14		2	3	5		2	1				11.8		3	6	4												
22	15			2	5	4	6	2	3			12.0	1	2	11	6	1	1										
9	16		1	2	1	2	3					10.8		5	3	1												
2	17						1	1				13.0			1	1												

variation of the several groups, whether they be observed from the standpoint of averages or distribution. If, however, the two groups be combined and the columns be made to include 2 mm range in leaf width as is done in table 91 (see row for (3×34) F_3), we see a slight but definite decline in variability toward the wider-leaved groups.

Summary; width of leaf

In the 3×34 cross, the parents had essentially the same leaf width. The average of the F_1 was a little below either parent, the F_2 exhibited quite marked differences in the means of the different F_2 cultures but the average of the whole F_2 population was the same as that of the wider-leaved parent. In the F_3 the leaves of the hybrids averaged wider than those of either parent and there were again considerable differences in the means of the different hybrid cultures (see table 89). The differences observed, however, are not genetic differences, as is shown by the fact that there was no correlation whatsoever between the leaf width of the F_2 selected parents and the mean leaf width of their offspring (see table 88). In other words, the progeny of the different variants of the F_2 gave results such as would come from the fluctuants of a pure race. We may therefore justly conclude that so far as leaf width was concerned, the 3×34 hybrids formed a pure race. This,

however, does not mean that these hybrids really formed a pure race in all characters for we have already seen that they segregated in both height and date of heading. A plant may easily be homozygous for one character and heterozygous for a number of others. We may assume therefore that the 3×34 hybrids received the same set of leaf-width factors from both parents. In the subsequent discussions of leaf width this group will be considered as a single pure variety.

Before proceeding with the summary and discussion of the other crosses we may first seek to discover whether or not a cause such as we found to suppress variability in the tall cultures of wheats was also operative in reducing variability in the wider-leaved cultures. Table 91 brings together all available data bearing on this point. The horizontal rows contain the data from plants or groups which were supposed to be genetically equivalent so far as leaf width is concerned.

The results obtained in table 92 are remarkably uniform and exhibit without doubt some general cause suppressing variability in the broader-leaved cultures. The nature of this suppression factor is not yet determined. Three possible explanations are suggested as follows:

(1) Can it be that the coefficient of variation is not a proper measure of the variability of quantitative characters in biology?

(2) Is it possible that even pure lines of wheat are still somewhat heterozygous and that the taller cultures are more homozygous than the others?

(3) Can there be some physiological limitation of growth in the higher classes which restricts the full development or expression of the plus combinations of factors?

The writer is inclined to attribute this suppression factor to a combination of suggestions (1) and (3). If a car be moving at rate A and we apply an additional force, say $F+m$, which gives an additional speed say $A+n$, it will require more force than $F+2m$ to give it a speed of $A+2n$.

The effect of a factor, environic or genetic, for increasing size, is probably much less in a combination which tends to produce a variant above the racial mean than in combinations, the product of which falls below the mean. We should have, as it were, a telescoping of variability in cultures with higher means. It is possible therefore that a better measure of the variability of quantitative characters would be a coefficient derived by dividing the standard deviation by some fractional

power of the mean, thus $C_1 = \frac{\sigma}{M^x}$ where x is a quantity less than 1.

Returning to the macaroni—bread wheat crosses we remember that

TABLE 91

Correlation of average leaf width of culture and the coefficient of variation of the same in pure lines and genetically equivalent groups.

Culture		Total number	Leaf width in millimeters							
			9 10	11 12	13 14	15 16	17 18	19 20	21 22	
Pure No. 1.....	No. of cultures	16			2	8	4	2		
	Average C. V.				11.0	10.6	9.9	9.0		
Pure No. 3.....	No. of cultures	4			2	2				
	Average C. V.				11.8	10.5				
Pure No. 34.....	No. of cultures	6		1	4		1			
	Average C. V.			12.0	11.3		11.0			
Pure No. 35.....	No. of cultures	9				2	3	3	1	
	Average C. V.					14.5	13.7	14.7	8.0	
(1 × 35) F ₂	No. of cultures	37			5	31	1			
	Average C. V.				30.6	29.1	27.0			
(1 × 3) F ₂	No. of cultures	6			3	3				
	Average C. V.				26.7	25.0				
(3 × 34) F ₂	No. of cultures	6				3	3			
	Average C. V.					10.7	9.0			
(3 × 35) F ₂	No. of cultures	18					16	2		
	Average C. V.						13.6	12.5		
(3 × 34) F ₃	No. of cultures	99			18	63	18			
	Average C. V.				12.0	11.8	10.3			
F ₂ cultures from tall F ₂ plants having smooth seeds (parent- like) (1 × 35) F ₃	No. of cultures	36			8	12	14	2		
	Average C. V.				19.1	17.9	15.6	14.5		
F ₂ cultures from tall F ₂ plants having wrinkled seeds (F ₁ - like) (1 × 35) F ₃	No. of cultures	28		3	6	16	2		1	
	Average C. V.			30.0	26.5	22.8	20.5		13.0	
F ₂ cultures from tall F ₂ plants having smooth seeds (parent- like) (1 × 3) F ₃	No. of cultures	9		1	2	3	3			
	Average C. V.			19.0	16.5	11.3	10.0			
F ₃ cultures from tall F ₂ plants having wrinkled seeds (F ₁ - like plants) (1 × 3) F ₃	No. of cultures	9	1	4	3	1				
	Average C. V.		20.0	27.8	26.7	25.0				

the F₁ had wide leaves and wrinkled grains. The average leaf width of the F₂ was markedly below that of either parent but there were some F₂ plants having leaf widths as great or greater than the parental means. These wide-leaved F₃ plants were of three types, viz., (1) some had wide leaves and smooth grains (parent-like), (2) some had wide leaves and wrinkled grains (F₁-like) and a few had wide leaves and partially wrinkled grains (of uncertain classification). Now since the average

of the F_2 was below that of the parents and the variability was much above the parental variability, we should expect the F_1 -like F_2 plants to give F_3 cultures low in mean leaf width and high in variability, whereas the parent-like F_2 plants should give F_3 cultures high in mean leaf width and low in variability. Now disregarding the wide-leaved F_2 plants with partially wrinkled seed (on account of difficulty of classification) we find the results shown in table 92.

TABLE 92

	(1 × 35) F_3			(1 × 3) F_3		
	Number of cultures	Mean leaf width	Average C. V.	Number of cultures	Mean leaf width	Average C. V.
F_3 cultures from wide-leaved smooth-seeded F_2 plants (parent-like)	36	16.1	17.1	9	15.4	12.9
F_3 cultures from wide-leaved wrinkled-seeded F_2 plants (F_1 -like)	28	14.9	23.8	9	12.4	26.2

No better agreement of the facts with the theoretical assumptions made, could well be expected. It is, of course, not here assumed that the parent-like F_2 plants were constituted genetically exactly like one or the other of the parents or that the F_1 -like F_2 plants were completely heterozygous in every particular in which the F_1 plants were heterozygous, but it is assumed that the genetic agreement is close enough to give marked similarity in form and hereditary behavior. Where a number of factors are involved, as there probably are here, it would be extremely difficult, probably impossible, to pick out plants from the F_2 by inspection, which were exactly like either the parents or the F_1 , genetically. This could only be done by judging the F_2 plants by the genetic behavior of their offspring. The facts developed seem to show that the wide-leaved F_2 plants fell into two groups, the one having a complete (or nearly complete) set of the factors from one or the other of the parental races, and that the other group contained plants which were heterozygous for all (or nearly all) of the characters in which the parents differed. Again therefore we have a situation where a complete double set of one or the other of the parental races or a complete (or nearly complete) single set from each of the two parents were able to

produce wide-leaved plants, but that the large majority of the new recombinations of parental characters resulted in less vegetative development.

Now referring to table 92 we find that even in the offspring of these two groups of wide-leaved F_2 plants the factor for suppressing variability was apparent, but it was not sufficient to mask the effect of differences in heterozygosity because in the one case (those of the F_1 -like parents) the means tended to be below that of the standard (pure line parents). Now when we turn to the 3×35 cross where the F_1 , F_2 and F_3 all had average leaf widths larger than the more narrow-leaved parent, the suppression factor was able entirely to offset the theoretically expected increased variability of the heterozygous cultures. If in accordance with the F_1 , the wide-leaved F_3 cultures were the more heterozygous and the more narrow-leaved the more homozygous we can easily see how the suppression factor might reduce the average variability of all of the F_3 cultures to a figure equal to or below that of the most variable parent especially in a case where the average of the leaf width of the F_3 cultures was equal to that of the wider-leaved parent.

One cannot here assume the formation of a single new blended race, for table 75 shows segregation in the F_2 with the formation of many distinct races in F_3 , and moreover, in spite of the suppression factor and the fact that the F_2 had a larger mean than the F_3 , the average variability of the F_3 was less than that of the F_2 (compare tables 76 and 78).

According to Mendelian expectation, the parental types of individuals in F_2 and culture means in F_3 were recovered in all cases. In 1×35 , recombination formed individuals in F_2 and a number of cultures in F_3 whose means were significantly beyond, both above and below, the range of either parent. In 1×3 the range of individuals in F_1 and of means of cultures in F_3 were significantly below, but not above, the parental ranges. In 3×35 the range of individuals in F_2 and means of cultures in F_3 were not significantly above or below the parental ranges.

In the macaroni—bread wheat crosses the average variability of the F_2 and F_3 generations were markedly above that of the parents but in the F_3 many cultures were secured which were as little variable as either parent. In no case was there a single F_2 culture, however, which had as low a variability as the most variable parental culture.

The variability of the bread wheat crosses has already been discussed with sufficient fullness.

The segregation of simple Mendelian unit factors appears to suffice to

explain all of the facts so far observed in the inheritance of leaf width in the wheat hybrids here discussed. No attempt has been made to determine the number of factors but the supposition is that there are several.

GENERAL SUMMARY

Detailed summaries of the three characters, date of first head, height, and width of leaf, may be found on pages 27, 52 and 87, respectively.

The F_1 of the macaroni ~~x~~ bread wheat crosses developed normally and were in every case equal or superior to the mean of the parents in vegetative vigor and they were no more variable in size characters or time of maturity than were the pure races. We may therefore conclude that a single complete set of macaroni wheat characters with a complete single set of bread wheat characters (the maximum of heterozygosis between the two varieties) will produce a perfectly normal plant.

In the second generation, on the other hand, many of the seeds would not germinate and those germinating produced plants differing in vegetative growth from those which were more vigorous than either parent to such as never got beyond the rosette stage. Moreover those which made a normal vegetative development exhibited every degree of sterility from completely sterile plants to those entirely normal in seed production. It would appear, therefore, that these facts alone refute any idea of blending inheritance, for if blending had taken place in the F_1 , sterile or vegetatively deficient plants would be no more likely to occur in the F_2 than in the F_1 . Hence we are compelled to predicate segregation and recombination in these quantitative characters. There is nothing to indicate even partial blending in any of the factors concerned.

In the use of the coefficient of variation as an indication of heterozygosity in hybrids involving quantitative characters, care should be exercised to make due allowance for the fact that races with high means resulting from increased vegetative growth, have their variability limited or reduced by the apparent law that size factors are more effective in producing variability in combinations tending to produce a result below the mean of the hybrid population than in combinations which tend to exceed this mean.

The suppression of variability in cultures with high means applies to pure as well as hybrid cultures. It appears to be a telescoping of variability as the mean approaches the upper physiological limit of growth rate for the species concerned.

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HEREDITY, VARIATION, AND THE APPEARANCE OF DIVERSITIES DURING THE VEGETATIVE REPRODUCTION OF *ARCELLA DENTATA*¹

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INTRODUCTION

The problem

In the vegetative reproduction of rhizopod Protozoa conditions for the study of heredity, variation, and the method of evolution are more

¹ This work was done at the Zoölogical Laboratory of the JOHNS HOPKINS UNIVERSITY. The writer is indebted to the members of the Zoölogical Department for many courtesies, and is especially grateful to Professor H. S. JENNINGS for his valuable counsel.

simple than in any other animals. The body of *Arcella* is a single cell, which is as simple as any animal organism it is possible to obtain for such studies. During vegetative reproduction the two offspring at each division consist each of one-half of the parent. There are thus no heritable variations due to amphimixis, and if changes occur that are of evolutionary significance they may be easily recognized. During the past four years several reports have appeared, notably those of MIDDLETON (1915), JENNINGS (1916) and ROOT (1918), that cast some doubt upon the conclusions of earlier workers, that heritable diversities do not occur within a "pure line," that is, that the genotype is constant. Work of this sort is crucial, since it tests one of the requirements of evolution, i.e., that heritable diversities should appear that are not due to the mixture of factors from several individuals. The present contribution adds one more species of Protozoa in which there is an apparent inconstancy of the genotype during vegetative reproduction.

Advantages of Arcella for genetic studies

Arcella dentata was chosen as the material for this investigation because it possesses an unusually large number of favorable characteristics (fig. 1.)

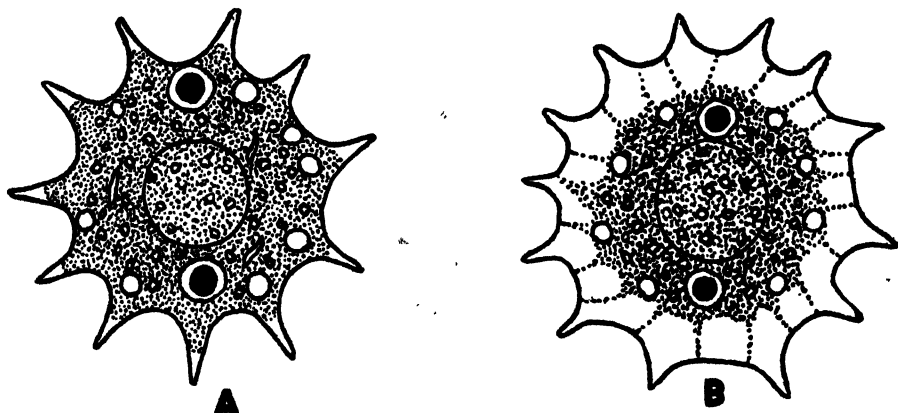


FIGURE 1.—A, aboral view of a typical specimen of *Arcella dentata* with 11 spines and a diameter of 116 microns. Imbedded in the cytoplasm (dotted) are 2 nuclei, a number of contractile vacuoles, and smaller food material. $\times 310$.

B, aboral view of a specimen just after division. This specimen had 13 spines and measured 138 microns in diameter. $\times 310$.

(1) It multiplies vegetatively and rapidly so that many generations may be obtained in a short period of time.

(2) It possesses definite morphological characters that are easily measured and not modified by growth.

(3) Environmental factors have no influence upon these characters after division is completed.

(4) Its characters are heritable but variable.

(5) The shell may be preserved easily, thus furnishing a permanent record.

(6) It is large enough to be conspicuous when placed under a Greenough binocular.

(7) The young are much lighter in color than their parents and hence there is no danger of confusing parent and offspring.

(8) The examination of living specimens is simple, because they attach themselves by their pseudopodia to the substratum and move very slowly.

(9) The almost transparent shell reveals the nuclei, chromidia, contractile vacuoles and other bodies within the living animal—especially in young specimens.

(10) Individuals will withstand severe operations and halves and even quarters will survive and reproduce provided they contain a nucleus.

Characters studied

The characters reported upon in this paper are (1) the number of spines, and (2) the diameter of the shell, measured between the bases of the spines. Other characteristics available for study are the size and shape of the spines (figure 5), the rate of fission, and the color of the shell.

Cultural methods

Arcella dentata, according to LEIDY (1879) and others, is rare when compared with *A. vulgaris* and *A. discoides*; nevertheless, an abundant supply was obtained from the lily pads in a pond on the campus of the JOHNS HOPKINS UNIVERSITY at Homewood, Baltimore.

The cultural methods used were similar to those employed by JENNINGS (1916) in his work on *Diffugia*. Water plants from nearby ponds were brought into the laboratory and the diatoms, algae, and ooze covering them were washed off by shaking in a bottle. The coarser materials were strained out and then the water containing the finer particles was transferred with a pipette to concavities in hollow-ground slides. As the work progressed, it was found that the *Arcellas* were not so apt to become covered by sediment if the culture slides were prepared from three to twelve hours before needed. During this time the particles sink

to the bottom, forming a fine film upon which the animals creep about and feed. It was also discovered that the Arcellas were more active and multiplied more rapidly if the pond water was diluted with an equal amount of distilled water. Thus in each cavity were placed two drops of the pond water containing food particles and two drops of distilled water. The brownish Arcellas are quite distinct against the grayish film of sediment on the bottom of such a culture, and there is no danger of contamination due to the inclusion of "wild" specimens that might occur in the food material.

New cultures were prepared, on the average, every three days, and the Arcellas were transferred to them with a capillary pipette. The cultures were examined almost every day and the young were removed to separate slides soon after they were produced. The number of the individual was then written with pencil on the rough surface of the slides. The cultures were not covered with cover glasses but were kept from drying by placing them in moist chambers consisting of large stender dishes. Each of these dishes accommodated eleven slides, or twenty-two specimens.

Specimens were easily found in the cultures with the help of a Greenough binocular and were then more closely examined under a compound microscope with the use of a water immersion objective.

For keeping pedigree records the system used by JENNINGS was adopted. This system was described by JENNINGS (1916, p. 415) as follows:

"For keeping pedigrees, records are kept in card catalogues, a card being devoted to each individual. The system may be illustrated as follows: The original 'wild' individual is given a number—say 21. Its first progeny is called 21.1, its second 21.2, etc. The first progeny of 21.1 is 21.1.1, its next 21.1.2, etc. Thus in later generations we have cumbrous labels such as 326.1.4.2.2.3.2.1.2.2.2.1.1.2. These labels must be written on the slide and its corresponding card. On the card is written a brief description of the individual, including the number of spines, and any peculiarity that will distinguish it from its progeny. Whenever an individual reproduces, that fact, with its date, and the number of spines of the progeny, is entered on the card of the parent, and a new card is written for the progeny. The long labels become troublesome, but each gives the full pedigree and relationship of the individual, and with the card records of all, the entire history may be reconstructed"

It was found desirable in certain cases to substitute for some of the longer labels one or more letters, such as **A**, **B**, **ED**, **SS**, etc.

Fission in Arcella

The process of fission in *Arcella* has not been described in detail and some of the stages were not observed during the course of this work. When ready to divide enough cytoplasm is protruded from the mouth of the shell to secrete a new shell; this cytoplasm lies against the substratum beneath the parent shell and thus the process of shell formation can not be observed from above. It seems probable that the spines of the shell are secreted around pseudopodia which extend out from this cytoplasmic mass. A number of specimens were examined after the new shell had been secreted but before division had taken place. In every case most of the cytoplasm was in the parent shell and only a small plug of cytoplasm extended into the new shell as shown in side view in figure 2. Cytoplasm then gradually flowed into the new shell until each shell contained approximately an equal amount. The cytoplasmic strand extend-

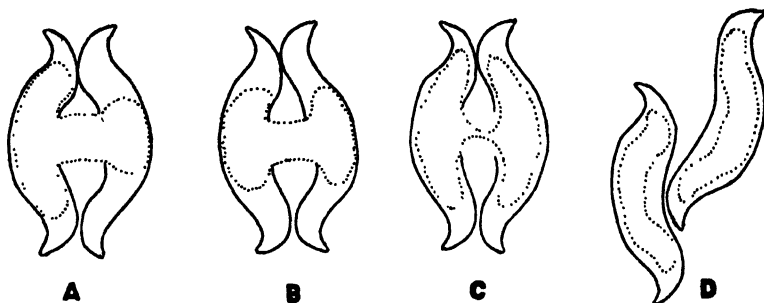


FIGURE 2.—Side views of division stages of *Arcella dentata*. The parent is at the left; the offspring at the right. The extent of the protoplasm is indicated by the dotted lines. The intervals between the stages are as follows: between A and B, 7 minutes; B and C, 3 minutes; C and D, 10 minutes. $\times 207$.

ing between the mouth openings of the two shells then grew thinner and finally broke and the parent and offspring moved apart sideways. There was no immediate swelling of the cytoplasm within the shells, which were therefore only about half full, as shown in figure 1B.

Parent and offspring

The method of division of *Arcella* with respect to the shell, presents several difficulties not encountered in the study of shellless Protozoa. When it divides, the protoplasm apparently divides into two equal parts, i.e., the living substance of the parent passes over into that of the two daughters, but whereas one of the daughters is provided with a new shell, the other continues to live in the parental shell. Throughout this

paper I have referred to the latter as the parent and the former as the offspring. If a specimen has 12 spines, this number must be due to two factors, (1) the hereditary constitution of the protoplasm of its parent and (2) the environmental conditions during or preceding the formation of its shell. It is thus safe to conclude that the number of spines it possesses is a good index of its hereditary constitution, since it contains one-half of the parental protoplasm that secreted the shell and the environment was kept relatively constant. However, we have found that the young often vary from the parent in spine number and the problem thus arises as to whether the protoplasm of a 12-spined parent that has just given rise to a 10-spined offspring really corresponds to the shell in which it is confined or is a "square peg in a round hole." On this account a number of correlation tables were made up from data of the immediate or first progeny of each parent. When these tables were compared with those in which later offspring were included, it was found that the results were so nearly identical that no further attention was paid to this apparent difficulty. It seems probable that heritable diversities do actually appear among the progeny of a single individual, but the diversities are so small and the number of offspring belonging to other than the first division that were recorded were so few that very little difference results from their inclusion in correlation tables.

Counting generations

A second difficulty that arises because of the persistence of one-half of the parental protoplasm in the parental shell at the time of division is the counting of generations. This is of some importance since we measure the "permanency" of a characteristic in part by the number of generations through which it persists. Should the second offspring from a "parent" be considered as a member of the f_1 or of the f_2 generation, and should the parent after each fission be considered as belonging to the same generation as its last offspring? The accompanying parts of a pedigree (figure 3, A, B) show that such a parent may be considered a member of either generation, but really belongs to the f_2 generation. For convenience, however, all numbers of generations mentioned in this paper, unless otherwise indicated, have been counted according to the first method. It is a very simple matter to determine the number of the generations according to either method from the system of labelling employed. For example the specimen designated by the numerals 58.1.1.1.4.2.1.1.1.3.1.3.1.2.4 belongs to the fifteenth generation according to the first method, this answer being found by counting the number of nu-

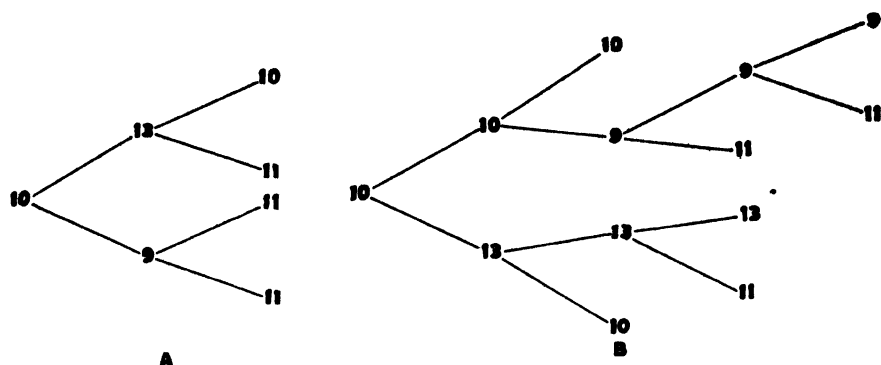


FIGURE 3.—Diagrams illustrating two methods of counting generations in *Arcella*. The diagram at the left, A, shows that the original parent with 10 spines gave rise successively to 2 offspring with 13 and 9 spines, respectively, and that each of these in turn gave rise to 2 offspring, the one with 13 spines to a first with 10 spines and a second with 11 spines, and the one with 9 spines to 2 successive offspring with 11 spines each.

In the diagram at the right, B, the parent after division is in each case shown as belonging to the same generation as the offspring.

merals after the family number, 58; or belongs to the twenty-seventh generation according to the second method, this answer being found by adding the same numerals together.

Preservation of specimens

It was found impossible in the time available, to preserve all the specimens, but a large proportion of them were saved for further study if this was found to be necessary. This was accomplished by transferring each individual to a separate drop of glycerine on a glass slide. One slide will hold without crowding about eight isolated specimens. The labels were written with India ink on the slides which were then placed in upright boxes where they keep for months without drying.

Length of life and number of progeny

No attempt was made to determine how many offspring may be produced by one parent nor how long a single parent will live. However, in looking over the records, it was found that specimen 58.1.1.1.1.1.2.1.1.1.1.1.2.2.1.1.1.2.1 was kept from December 20, 1917, to January 25, 1918, during which period it produced 18 offspring, and specimen 58.1.1.1.4.2.1.1.1.1.3.1.1.1.1 was kept from December 7, 1917, to January 24, 1918, and gave rise to 21 progeny. There seems to be no reason why

these specimens might not have continued to live and reproduce indefinitely.

Computations

In the tables presented in this paper the coefficients of correlation were determined by means of the formulae used by JENNINGS (1916, pp. 416-417). The actual pedigrees, however, are perhaps better evidence than correlation tables of what has really occurred, and as many parts of pedigrees have been included as seemed advisable.

The specimens that form the basis of this report were obtained during a period of 174 days. The average number of generations studied was 69 and the average length of a generation was 2.5 days. In all, measurements of 6474 specimens are recorded. Of these 5557 belonged to the single family No. 58; 746 were secured from 70 small families; and 171 were collected directly from the pond.

VARIATIONS IN SPINE NUMBER AND DIAMETER OF SHELL IN A "WILD" POPULATION

The principal problem that this investigation was designed to solve is, Do variations in heritable characteristics that may occur in the progeny of a single specimen reproducing vegetatively lead to temporary or permanent diversities that would account for the diversities present in a "wild" population?

We must, of course, determine first what the variations are in such a "wild" population. Accordingly a large number of specimens of *Arcella dentata* were collected from various parts of the pond where they were

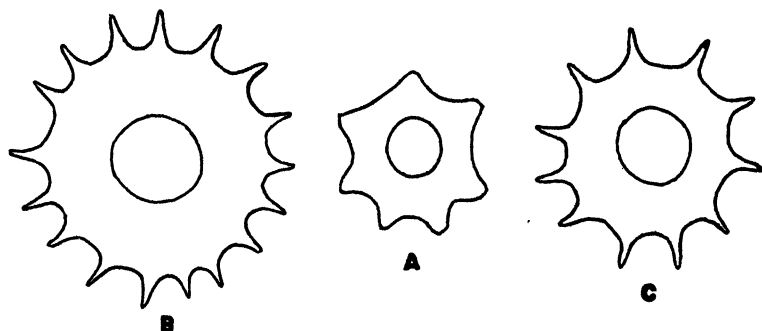


FIGURE 4.—Three specimens from a "wild" population of *Arcella dentata*: A was the smallest specimen found, possessing 7 spines and a diameter of 73 microns, B was the largest, with 17 spines and a diameter of 150 microns, C was in the modal class and near the mean, with 11 spines and a diameter of 116 microns. $\times 207$.

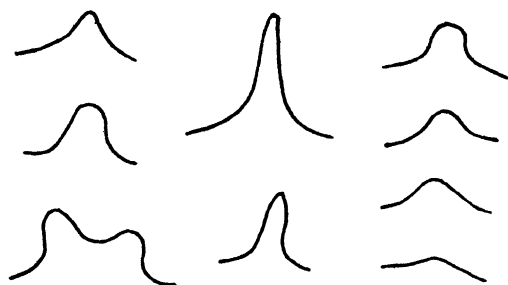


FIGURE 5.—Some variations in the sizes and shapes of the spines of *Arcella dentata*. $\times 420$.

present in great abundance. These were found to vary in size, and in number and length of spines. Some of these variations are indicated in figures 4 and 5. The extremes with respect to spine number were 7 and 17; and those with respect to diameter of the shell were 73 microns and 150 microns. No study was made of the variations in the shape or size of the spines, but such variations were incidentally noted and some of them are shown in figure 5.

TABLE I

Correlation table for spine number and diameter of the shell in a "wild" population of *Arcella dentata* collected from a pond at Homewood, Baltimore, on Sept. 20, 1917. The unit of measurement is 4.3 microns. Correlation, $.325 \pm .060$.

	Spine number							
	7	8	9	10	11	12	13	
23	1							1
24			1	2	1			4
25			2	12	4	1	1	20
26			3	12	10	3		28
27			4	12	7	2		25
28			3	4	5	5		17
29				2	1			3
30								0
31								0
32								0
33						1	1	2
	1	0	13	44	28	12	2	100

Table I shows the variations in spine number and diameter of the shells of 100 wild specimens taken at random from a large series collected on September 20, 1917, from the pond at Homewood and figures 6 and 7 give the same data in the form of curves. As regards spines, the mode is 10 and the mean 10.42, and for diameter the mode is 26 units and the mean 26.51 units. Specimens were found with spine numbers

of 8, 14, 15, 16 and 17, but these did not happen to appear in this sample of 100. A coefficient of correlation of $.325 \pm .060$ shows that there is a significant relation between spine number and diameter in these or-

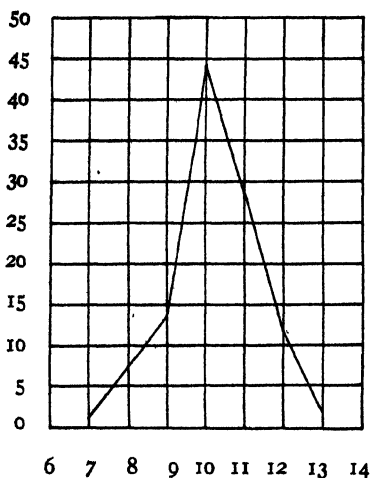


FIG. 6

FIGURE 6.—Curve showing the distribution of spine numbers of 100 "wild" specimens of *Arcella dentata* collected on Sept. 20, 1917, from a pond at Homewood, Baltimore. The ordinates are numbers of specimens and the abscissae numbers of spines.

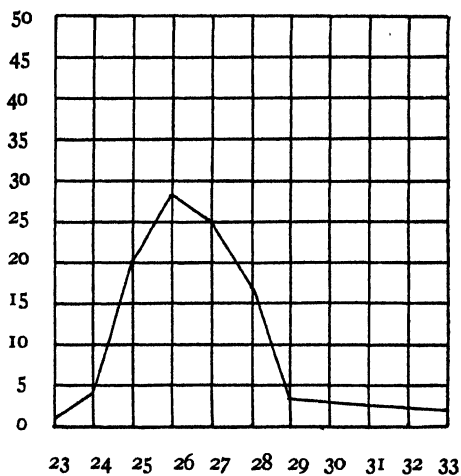


FIG. 7

FIGURE 7.—Curve showing the distribution of diameters of the 100 specimens recorded in figure 6. The ordinates are numbers of specimens and the abscissae diameters. The unit of measurement is 4.3 microns.

ganisms. Frequently irregular specimens were encountered (figure 12) principally among those with a low spine number; pedigree work indicates that many of these irregularities are due to environmental factors. They undoubtedly account for the high mean diameter of 26.46 of the group of 13 specimens with 9 spines shown in table 2. With the ex-

TABLE 2

Mean diameters of 100 "wild" specimens of *Arcella dentata* of given spine numbers. The unit of measurement for diameter is 4.3 microns.

Number of specimens	Spine number	Mean diameter
1	7	23.00
13	9	26.46
44	10	26.22
28	11	26.50
12	12	27.50
2	13	29.00

ception of this group, the mean diameter of the specimens measured increases gradually with the increase in spine number, exhibiting a marked correlation between these two characteristics.

Conclusion

The variations, therefore, in a random sample of 100 "wild" specimens are from 7 to 13 in spine number and from 23 to 33 units in diameter of shell, each unit being equal to 4.3 microns, and there is a marked correlation between the number of spines and the diameter of the shell.

HEREDITY AND VARIATION IN SPINE NUMBER IN FAMILIES DERIVED FROM SINGLE SPECIMENS BY VEGETATIVE REPRODUCTION

The next questions to be answered are, What kinds of variations occur during vegetative reproduction and to what degree are they inherited? Are heritable characteristics similar or different among families ("pure lines") descended from different "wild" specimens? To what degree are these heritable characteristics correlated in each family? Are there in nature a number of diverse families with regard to a given characteristic and are the several measurable characteristics correlated or independent in the various families? How many diverse families are there?

One hundred and forty-nine living specimens were selected from collections of wild animals, mostly taken on September 21 and 22, and November 23, 1917, and isolated on culture slides. These specimens were selected on the basis of diameter and spine number so that a beginning might be made with animals differing widely in these characters, as well as with those nearer the modal condition. Of the 149 specimens, 59 gave rise to small families and 12 to larger families. All of these, except one, either died out or were killed off because they could not be taken care of; the one family, number 58, was kept, being selected for further work. Tables 3 to 20 include only data regarding spine number.

Table 3 shows the distribution of the variations in number of spines within twelve of the larger families obtained. These are arranged according to the mean spine number beginning with the lowest mean. The range is from 10.40 to 14.07. A large interval occurs between the mean 11.21 of family 41 and that of 13.71 of family 62. Probably a larger number of families would supply means that would fill up this gap. Possibly, however, families 62, 109, and 83 should be considered as belong-

TABLE 3
Distribution of the variations in number of spines within twelve families.

Number of family	Number of spines										Total number of specimens	Mean spine number	Coefficient of correlation
	7	8	9	10	11	12	13	14	15	16			
18	1	1	4	8	11	5					30	10.40	.004 \pm .123
85		2	5	12	14	6					39	10.43	-.158 \pm .105
80		1	3	11	9	2	2				28	10.50	.013 \pm .013
30			22	11	9	5	11				58	10.51	.279 \pm .082
79		4	2	11	10	11					38	10.57	.032 \pm .001
81		1	1	8	17	5	1				33	10.81	.085 \pm .117
17		2	17	31	39	45	12	3			149	11.04	.062 \pm .055
59			3	2	7	6	2				20	11.10	.039 \pm .151
41		4		1	22	8	4	2			41	11.21	-.176 \pm .102
62			1	2	2	2	4	11	9	4	35	13.71	.725 \pm .054
109					1	2	4	11	10		28	13.96	.183 \pm .128
83						5	7	13	10	5	40	14.07	.312 \pm .093
											539		

ing to a distinct race. In only three families, 30, 62, and 83, is there any evidence of correlation between the spine numbers of parents and offspring.

Figure 8 gives the curves for four families separately and for twelve

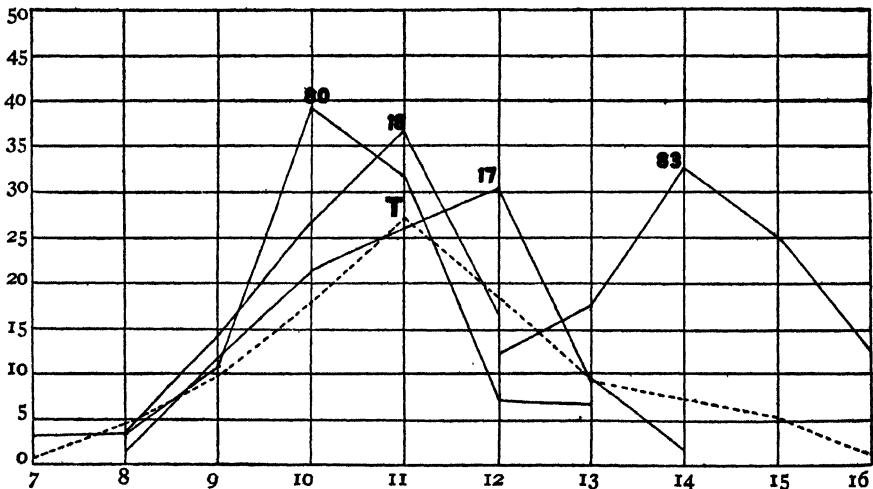


FIGURE 8.—Curves showing the distribution of the variations in spine number within four small families and for the total, T, of 539 specimens belonging to twelve families. The number with which each curve is marked is the designation of the family. The numbers of specimens in each family are shown in table 3. The ordinates are percentages; the abscissae, spine numbers.

families combined. Figures 9 and 10 are parts of the pedigrees of two of these families, showing quite clearly the great difference between them in spine number.

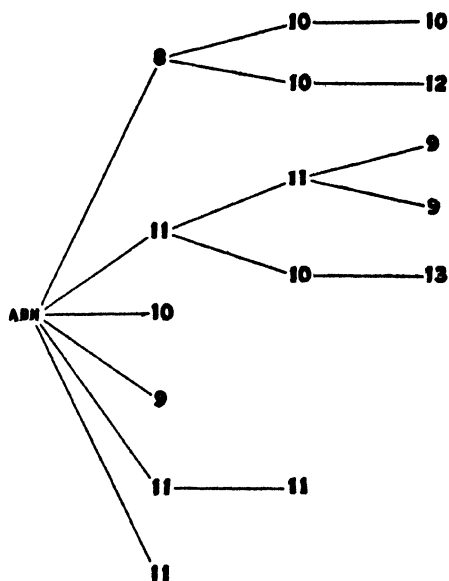


FIG. 9

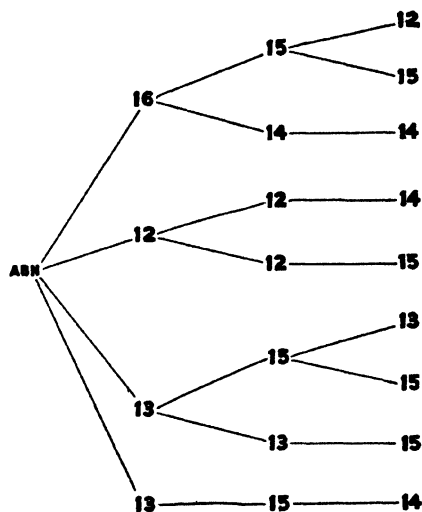


FIGURE 10

FIGURE 9.—Part of the pedigree of family 80 showing the number of spines of specimens belonging to the first 3 generations. The progenitor of this family (ABN; see fig. 12, A) was not normal and its abnormality was not inherited. It was selected for breeding purposes in order to determine whether or not abnormalities in *Arcella dentata* are inherited.

FIGURE 10.—Part of the pedigree of family 83 showing the number of spines of specimens belonging to the first three generations. This pedigree should be compared with that shown in figure 9. The abnormal progenitor of family 83 (see fig. 12, B) gave rise to normal offspring.

In all correlation tables the results of every fission were tabulated. Thus, when a specimen with 12 spines gave rise to one with 10 spines, the latter was recorded opposite number 10 in the parent column 12. Each offspring is counted but once as an offspring.

Table 4 presents the correlation for the inheritance of spines in the 70 small families taken together. This table consists of two principal sets of data; (1) the center of the table is occupied largely by offspring descended from specimens collected mostly on September 21 and 22, 1917. These have a mean spine number of a fraction less than 11.00. (2) The lower right-hand corner contains principally the descendants

TABLE 4
Correlation table for parents and offspring with respect to spine number within 70 small families. Correlation, $.626 \pm .015$.

Progeny	Parents																
	7	8	9	10	11	12	13	14	15	16	17						
7						1											1
8		2	1	1	3	4	1										12
9	1	1	12	11	13	9	1										48
10		9	12	28	40	36	10	1									136
11		9	16	37	44	44	9	4	1								164
12		2	5	17	38	25	25	7	4								123
13		2	3	3	9	17	24	11	9								78
14		1		1	4	24	20	29	18	6							103
15					2	11	14	11	10	12	1						61
16						2	1	4	8	4							19
17								1									1
	1	26	49	98	153	173	105	68	50	22	1						746

of very large specimens with many spines collected on November 23, 1917. This table shows that a high correlation ($.626 \pm .015$) exists between the spine numbers of parents and progeny in this large population containing progeny derived from 70 "wild" specimens.

In table 5 is given the mean spine number for the 746 offspring produced by parents with different numbers of spines within the 70 families studied. It is clear that the spine number of the progeny approaches more closely that of the immediate parents than it does the mean of the race.

Among the small families that were studied were several whose curves

TABLE 5
Mean spine numbers of 746 progeny of parents of given spine numbers within 70 small families.

Number of specimens	Spine number of parents	Mean spine number of progeny
1	7	9.00
26	8	10.69
49	9	10.42
98	10	10.72
153	11	11.00
173	12	11.66
105	13	12.75
68	14	13.72
50	15	14.12
22	16	14.90
1	17	15.00

of distribution of spine numbers almost coincide. This, of course, might happen with families derived from specimens taken from widely separated localities, but in this case it seems probable that, since the specimens were all collected from a rather small pond and some of them from a single leaf, they were closely related and hence gave rise to families similar in the distribution of spine number.

Conclusion

Variations in spine number occur among the descendants of a single specimen during vegetative reproduction, and these variations are in part inherited. The hereditary constitution of different families obtained by vegetative reproduction from different "wild" specimens is different with respect to spine number. There is a marked correlation between parent and offspring with respect to spine number in three of the families (30, 62, and 83). The small differences between certain of the means, e.g., between those of the first five families listed in table 3, namely, 10.40, 10.43, 10.50, 10.51, and 10.57, indicate that, with respect to spine number, the heritable diversities between the different families may be extremely small and that a vast number of families exhibiting such heritable diversities may occur in a "wild" population. The large gap between the means 11.21 and 13.71 of families 41 and 62 would probably be bridged if a larger number of families were obtained, but this may represent a difference that might be considered of racial magnitude.

PERMANENT DIVERSITIES IN SPINE NUMBER AMONG THE DESCENDANTS OF A SINGLE SPECIMEN PRODUCED BY VEGETATIVE REPRODUCTION

At the end of 39 days from the time the rearing of the Arcellas was begun, their numbers had increased to such an extent that it was necessary to eliminate all but one family. The latter, family 58, was retained for the purpose of determining the degree of inheritance and the extent of diversities in a single large family, and to answer the following questions: Do variations that may appear in heritable characteristics in the descendants of a single specimen reproducing vegetatively lead to temporary or permanent diversities? May such diversities account for the heritable differences that appear between families obtained from "wild" specimens? In what way are such diversities produced?

The family derived from specimen 58 was saved for this work since its mean spine number was near the average of the species and its members seemed more viable than those of the other families. For convenience and also for the purpose of checking up environmental influence,

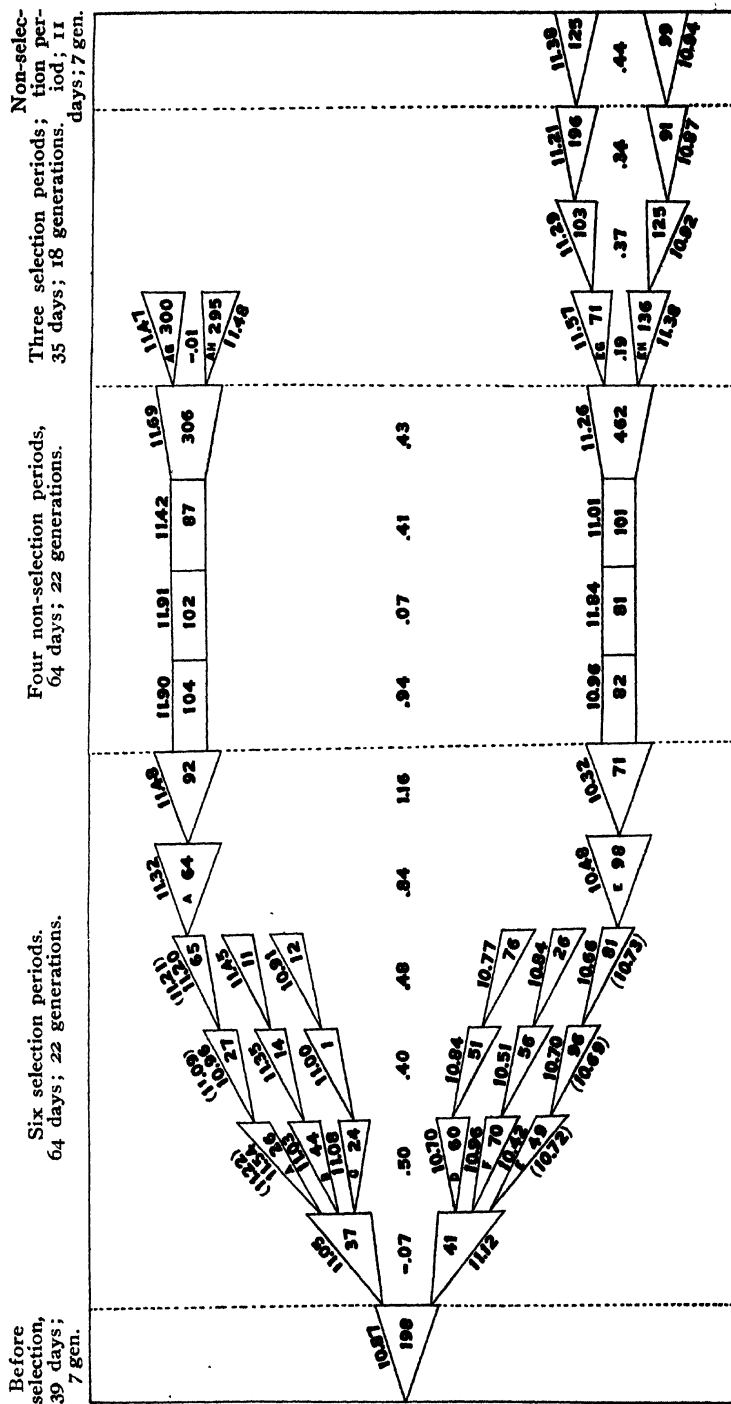


FIGURE 11.—Diagram showing the results of selection within the single family 58 for diversities in spine number. Each triangle or rectangle represents a period. The triangles are intended to indicate a gradual increase from one or a few selected individuals at the point at the left to a large number at the right. The rectangles indicate that the number of specimens was kept the same throughout the period. Within each triangle or rectangle is given the number of specimens studied during that period, and above each is given the mean spine number for the period. The upper series of triangles and rectangles give the data for the high line, and the lower series the data for the low line. Between the two series are the differences in mean spine number between the high and low lines. During the periods 2, 3 and 4, of the first series of selection periods, the high and low lines were made up of three branches each and labelled A, B, C and D, E, F, respectively. The means for the three branches taken together during these periods are given in parentheses at the bottom of the high line and below the triangles in the low line.

the experiment was divided into a number of periods. The length of these periods was regulated so that enough progeny were obtained in each to give significant means. The periods were also determined in part by the fact that only a few hundred specimens can be taken care of by one investigator and when this limit has been reached a selection must be made. It may be noted at this place that several sudden large variations occurred in several of the periods, but the specimens exhibiting them, and their descendants are not included in the data presented in this part of the investigation but are given later under a separate heading. The data for the periods are given in tables 6, 14, and 18 to 21, and are indicated in the diagram on page 110 (figure 11), reference to which will make the following account clear.

Data before selection was begun

The progenitor of family 58 (figure 13, **A**) was collected on September 22. It had 12 spines. Because of faulty cultural methods it died after dividing only once, and a number of the early members of the family died from the same cause (figure 14). Later, specimens were very seldom lost, although a few died, some dried because of the evapo-

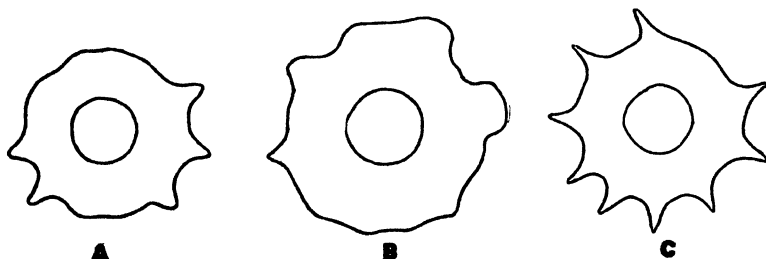


FIGURE 12.—Three abnormal specimens: **A** was the progenitor of family 80, and **B** of family 83; **C** apparently lacks one or two spines because of some mechanical obstruction in the environment. $\times 207$.

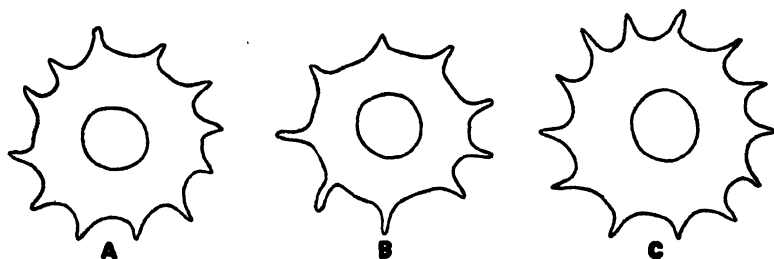


FIGURE 13.—Three specimens belonging to family 58. **A**, the progenitor of the entire family; **B**, a typical member of the low line; **C**, a typical member of the high line. $\times 207$.

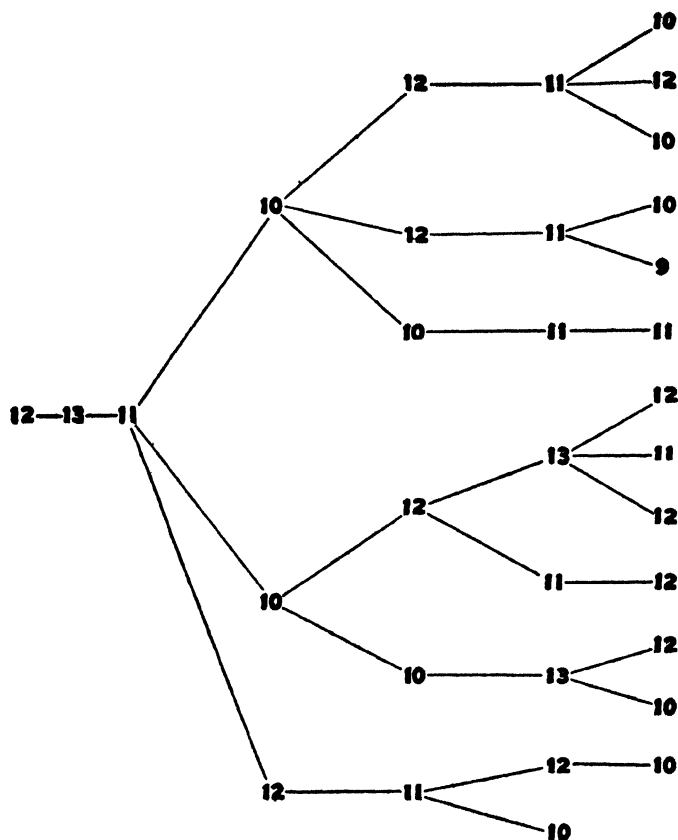


FIGURE 14.—The first part of the pedigree of family 58.

ration of the culture water, and several were lost during their transference from one culture to another. The first period was the longest and lasted for 39 days. During this time 198 offspring were produced with a mean spine number of 10.87. Table 7 gives the data for these 198 specimens. This table shows no evidence of correlation between parents and offspring, the coefficient of correlation being $-.027 \pm .048$.

First selection period

On November 1 selection was begun. All specimens with 12 or more spines were selected as a basis for the high line and all those with 10 or less for the low line. These and all of their offspring were kept. The specimens selected for both the low and high lines were placed in the same moist chambers; were given the same proportions of food and water; and were all changed at the same time. Environmental condi-

tions were thus almost exactly alike for all the specimens at any particular time during the course of the work. At the end of 9 days this period was closed. The diagram on page 110 and table 6 show that the 37 progeny of the high line had a mean spine number of 11.05, and the

TABLE 6

Family 58. Results of selection for number of spines during selection periods 1 to 6. (Nov. 1, 1917, to Jan. 3, 1918.)

Period	Number of progeny	Correlation	High line		Low line		Difference
			Number of progeny	Mean spine number	Number of progeny	Mean spine number	
1 (9 days)	78	.060 \pm .076	37	11.05	41	11.12	-.07
2 (25 days)	273	.220 \pm .039	94	11.22	179	10.72	.50
3 (9 days)	245	.186 \pm .042	42	11.09	203	10.69	.40
4 (8 days)	271	.185 \pm .040	88	11.21	183	10.73	.48
5 (7 days)	162	.403 \pm .044	64	11.32	98	10.48	.84
6 (6 days)	163	.512 \pm .039	92	11.48	71	10.32	1.16
64 days	1192					Mean difference .55	

TABLE 7

Family 58. Correlation table for parents and offspring with respect to spine number before selection was begun. Correlation, $-.027 \pm .048$.

		Parents					
		9	10	11	12	13	
Progeny	9		3	4	1		8
	10		9	19	15	2	45
	11	5	12	21	25	6	69
	12	1	16	19	14	6	56
	13		8	5	7		20
		6	48	68	62	14	198

TABLE 8

Family 58. Correlation table for parents and offspring with respect to spine number in the first selection period (Nov. 1 to Nov. 9, 1917).

Correlation .060 \pm .076.

		Parents						
		8	9	10	11	12	13	
Progeny	8	1					1	
	9	1					1	
	10	4	8	1	7	2	22	
	11	4	10	1	10	2	27	
	12	3	5	1	9	2	20	
	13	3			3	1	7	
		11	26	3	31	7	78	

41 progeny of the low line had a mean spine number of 11.12. The diversity obtained was thus in the wrong direction, the mean spine number of the low line being actually greater by .07 than that of the high line. Table 8 indicates an entire absence of correlation during this period.

This result may have been due to the small number of offspring used, but later work makes it seem more probable that the selection was not successful, because high- and low-spined specimens were picked out regardless of the condition of their immediate and more distant ancestors,—a method that JENNINGS (1916) has shown to be inefficient under similar conditions in *Diffugia corona*.

Second selection period

The method of selection was changed at this time (November 10) and specimens with 12 or 13 spines that had parents, grandparents or other close relations with a high spine number were selected as progenitors of the high line, and similarly, specimens with 10 or less spines whose near relatives possessed low spine numbers, were selected as progenitors of the low line; that is, selection was based on past performance. All parents and offspring were kept during this period which extended over 25 days, from November 10 to December 4. Difficulty was encountered in the rearing of the Arcellas at this time. Many of them ceased to divide and all of the specimens became entangled in the food material to such an extent that they had to be extricated with needles. Large numbers died and the entire family seemed doomed, when a simple remedy was discovered. This was the dilution of the pond water, with which the cultures were made up, with an equal amount of distilled water. This procedure was suggested to me by the fact that DR. VERNON LYNCH of the JOHNS HOPKINS MEDICAL SCHOOL, had previously found that the addition of distilled water to his cultures was of benefit in the rearing of Amebas. Division immediately began again and no more trouble was experienced throughout the entire winter and spring seasons.

During the second selection period the 94 progeny in the high line possessed a mean spine number of 11.22, and the 179 progeny of the low line, a mean spine number of 10.72, or a divergence of .50. The mean spine number of the high line was thus .35 above the mean of the family before selection was begun and that of the low line was .15 below this mean. Table 9 shows the degree of correlation of parent and offspring during this period.

It was found that at the end of the second selection period all progeny in the high line were descended from three specimens, and similarly, all

those in the low line were descended from three specimens. The branches to which these specimens had given rise were from this time on designated as **A, B, and C** in the high line, and **D, E, and F**, in the low line, and the data for these six branches are indicated in the diagram on page 110. It is worthy of note that the mean spine number of each of the

TABLE 9

Family 58. Correlation table for parents and offspring with respect to spine number in the second selection period (Nov. 10 to Dec. 4, 1917).

Correlation .220 \pm .039.

		Parents					
		8	9	10	11	12	13
Progeny	8			1			1
	9			5	4	5	14
	10		1	34	23	9	70
	11		7	40	43	20	112
	12		1	11	28	22	67
	13			1	3	4	9
		9	92	101	60	11	273

three subfamilies in the high line is greater than that of any of the three subfamilies in the low line, and that the means of all six subfamilies differ slightly from one another. Too much emphasis should not be attached to this but it suggests the possibility that these six subfamilies may represent six permanent heritable diversities in spine number.

Third selection period

At the end of the second selection period a few specimens were selected from each of these six branches on the basis of past performance and all parents and progeny were kept until the end of the period (9 days). As shown by the diagram on page 110, the three branches of the high line did not increase as rapidly as did those of the low line. During this period the mean spine number of the 42 offspring in the high line was 11.09, and of the 203 offspring in the low line, 10.69, giving a divergence of .40.

As during the second selection period, the mean spine number of each of the three branches of the high line is greater than that of any of the three branches of the low line. Table 10 shows the degree of correlation between parent and offspring during this period.

Fourth selection period

At the end of the third selection period a few specimens were selected from each of the three branches of the high line and from each of the

TABLE 10

Family 58. Correlation table for parents and offspring with respect to spine number in the third selection period (Dec. 5 to Dec. 13, 1917).

Correlation $.186 \pm .042$.

		Parents					
		8	9	10	11	12	13
Progeny	8	2					2
	9		5	13	4		22
	10	1	8	38	13	9	69
	11	2	13	44	20	18	101
	12	1	6	18	4	11	45
	13			4	1	1	6
		4	32	119	42	39	9
		245					

three branches of the low line, on the basis of past performance.

All these specimens and their offspring were kept during the period of 8 days. As indicated in the diagram on page 110, the mean spine number of each branch of the high line was higher than that of any branch of the low line. The mean of the entire high line was 11.21 and that of the entire low line 10.73, giving a divergence of .48. Table 11 shows the degree of correlation between parent and offspring during the fourth selection period.

Fifth selection period

At the end of the fourth selection period, two branches, **B** and **C**, were eliminated from the high line, and two, **D** and **F**, from the low line. As

TABLE 11

Family 58. Correlation table for parents and offspring with respect to spine number in the fourth selection period (Dec. 14 to Dec. 21, 1917).

Correlation $.185 \pm .040$.

		Parents					
		8	9	10	11	12	13
Progeny	8						
	9	1	3	9	3	3	19
	10	1	13	28	16	11	72
	11		19	43	23	23	113
	12		5	16	13	18	56
	13			1	2	6	11
		2	41	98	61	57	12
		271					

progenitors of the fifth period, a few specimens from branches **A** and **E** were selected on the basis of past performance.

Selection was also practiced *during* the course of the fifth period; all progeny in the high line with less than 12 spines were immediately elimi-

nated after being recorded and all specimens that persisted in giving rise to low-spined offspring were likewise eliminated; in a similar way all offspring in the low line with more than 10 spines were eliminated, and also all specimens that produced several high-spined progeny. All progeny were, of course, included in the data before they and their parents were eliminated. This method of selection was most effective. The mean spine number of the high line rose to 11.32, that of the low line decreased to 10.48, and the divergence increased to .84. Table 12 shows the degree of correlation between parents and offspring during this period, and figures 15 and 16 give parts of the pedigrees of the two lines at this time.

Sixth selection period

Selection at the beginning and during this period was carried on in

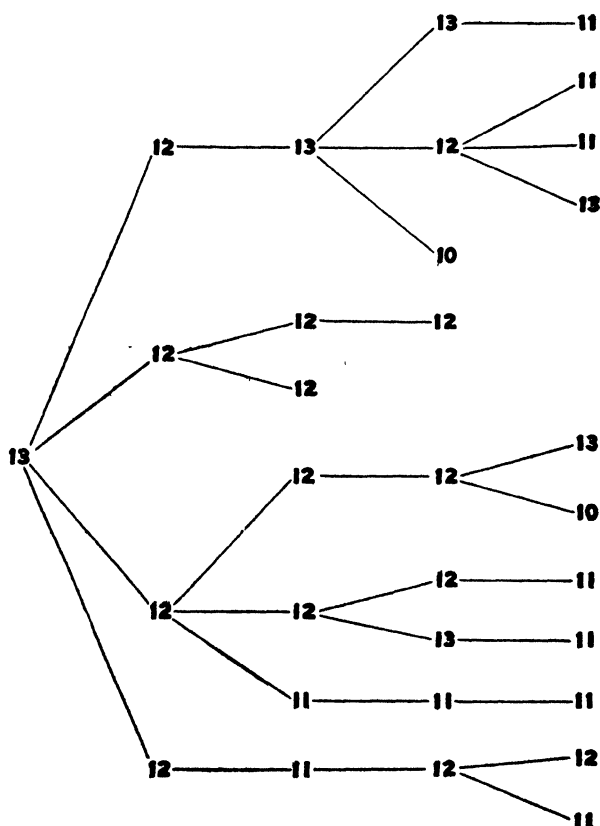


FIGURE 15.—Part of the pedigree of the high line of family 58 during the fifth selection period.

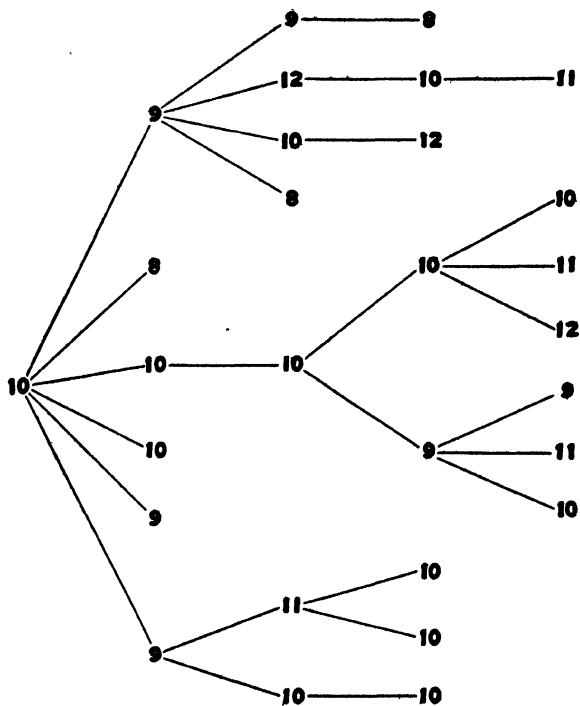


FIGURE 16.—Part of the pedigree of the low line of family 58 during the fifth selection period.

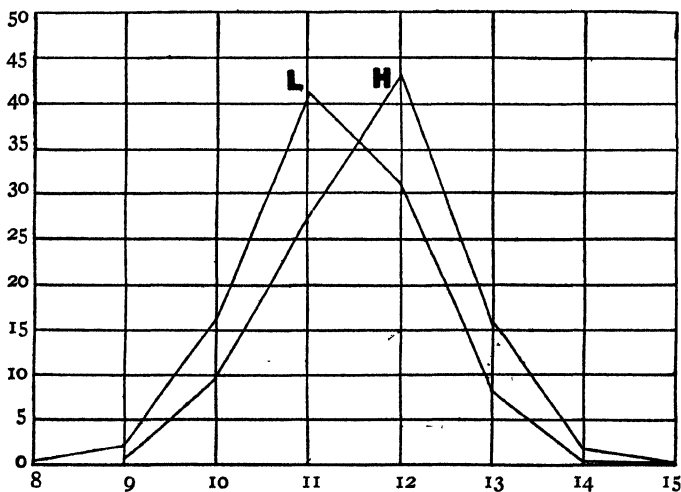


FIGURE 17.—Curves showing the distribution of the variation in spine number within the high and low lines of family 58 during the non-selection periods. The ordinates are percentages; and abscissae, spine numbers; H= the high line; L= the low line.

TABLE 12

Family 58. Correlation table for parents and offspring with respect to spine number in the fifth selection period (Dec. 22 to Dec. 28, 1917).

Correlation $.403 \pm .044$.

		Parents						
		8	9	10	11	12	13	14
Progeny	8		1	2	1			4
	9	1	6	1		1		9
	10	2	9	14	6	7	3	41
	11	1	3	20	18	28	5	75
	12	1	1	5	5	10	3	26
	13				1	5		6
	14					1		1
		5	20	42	31	52	11	162

the same way as during the fifth period. A rise in mean spine number to 11.48 in the high line, and a decrease to 10.32 in the low line gave a divergence of 1.16. Table 13 shows the degree of correlation between parents and offspring during this period.

Selection was discontinued at the end of the sixth period. The mean spine number had increased in the high line from 10.87, which was the

TABLE 13

Family 58. Correlation table for parents and offspring with respect to spine number in the sixth selection period (Dec. 29, 1917 to Jan. 3, 1918).

Correlation $.512 \pm .039$.

		Parents						
		8	9	10	11	12	13	14
Progeny	8		2	2				4
	9	3	2	2	1		1	9
	10	2	6	18	1	9	1	37
	11		5	15	4	19	12	56
	12		3	5	3	20	12	43
	13				2	7	3	13
	14						1	1
		5	18	42	11	55	30	163

mean of the family before selection was begun, to 11.48, a difference of .61, and had decreased in the low line from 10.87 to 10.32, a difference of .55. The average difference between the means of the high line and the low line during these six selection periods was .55.

Conclusion

It has thus been demonstrated that by means of selection, two lines can be obtained from a single specimen reproducing vegetatively, that differ in measurable, heritable characteristics. This difference may seem

slight but it is constant not only for the high and low lines, but also for the separate branches (**A, B, C** and **D, E, F**) into which these lines were divided, and hence is significant. The question now arises, Is this divergence temporary or permanent?

Non-selection periods

To test the permanency of the divergence obtained during the six selection periods of 64 days, a series of non-selection periods was inaugurated. Because it is impossible for one investigator to take care of more than a few hundred specimens at one time, the following method was used during the first three non-selection periods. Twenty-two representative specimens (the number that can be accommodated in one moist chamber) were taken from the high line, and 22 from the low line. Whenever these divided, the parent was eliminated and the offspring kept. Thus the number of specimens was kept down but no selection was practiced. Only immediate progeny obtained during the first three non-selection periods were thus recorded, but during the fourth period, several offspring from a single parent were included. The data obtained during the four non-selection periods are presented in figure 11, and in tables 14, 15 and 16. As the diagram on page 110 and table 14 show, there was an immediate decrease in the difference between the means of the high and low lines from 1.16 to .94 during the first non-selection period and to .07 during the second non-selection period. During the third period the difference increased to .41.

At the end of the third non-selection period, the high and low lines were allowed to expand; that is, *all* parents and offspring were kept. This was continued for 15 days and a very large number was obtained in

TABLE 14

Family 58. Spine numbers during the four non-selection periods (Jan 3 to Feb. 6, 1918).

Period	Number of progeny	High line		Low line		Difference
		Number of progeny	Mean spine number	Number of progeny	Mean spine number	
1 (6 days)	186	104	11.90	82	10.96	.94
2 (7 days)	183	102	11.91	81	11.84	.07
3 (7 days)	188	87	11.42	101	11.01	.41
4 (15 days)	768	306	11.60	462	11.26	.43

35 days 1325

Mean difference .46

TABLE 15

Family 58. High line. Correlation table for parents and offspring with respect to spine number in non-selection periods 1 to 4 (Jan. 3 to Feb. 6, 1918). Mean spine number of all (588) progeny, 11.94. Correlation, $.170 \pm .027$.

		Parents							
		8	9	10	11	12	13	14	15
Progeny	9			4		1			5
	10		2	10	18	17	6	2	55
	11		4	28	32	71	20	5	160
	12		2	30	62	114	43	6	257
	13		1	11	17	40	25	3	98
	14			2	1	5	3	1	12
	15					1			1
		9	85	130	249	97	17	1	588

TABLE 16

Family 58. Low line. Correlation table for parents and offspring with respect to spine number in non-selection periods 1 to 4 (Jan. 3 to Feb. 6, 1918). Mean spine number of all (737) progeny, 11.28. Correlation, $.197 \pm .024$.

		Parents							
		8	9	10	11	12	13	14	15
Progeny	8				1				1
	9			1	6	4	4	2	17
	10		2	8	30	47	27	7	121
	11			8	48	135	92	19	303
	12			2	50	82	72	21	229
	13				6	20	19	17	62
	14					1	1	1	3
	15						1		1
		2	19	141	290	215	67	1	737

each line; 306 in the high line and 462 in the low. Again a difference between the two lines appeared—this time of .43. Since the divergence between the high and low lines persisted throughout the 4 periods of non-selection, representing 18 generations, the conclusion was reached that a permanent divergence had been obtained.

It will be noticed that the mean spine number increased in both the high and low lines during the first non-selection period. Thus that of the high line during the sixth selection period was 11.48 and in the first non-selection period was 11.90, and that of the low line during the sixth selection period was 10.32 and in the first non-selection period 10.96. The mean spine number of the high line increased only by .01 during the second non-selection period; whereas that of the low line increased by .88. A decrease in both lines occurred in the third non-selection period, and an increase again in the fourth non-selection period. Several other

lines were being propagated at the same time and in these similar increases and decreases were noted during these periods. One of these lines consisted of specimens derived from a large *Arcella* collected on December 27, 1917, thus showing that these changes in spine number were not restricted to descendants of specimen 58. A similar increase in spine number was noted by JENNINGS (1916, p. 489) in *Diffugia corona*. The cause of these increases and decreases is not known but their appearance in all lines at the same time points to some environmental factor, probably the composition of the water or food. Some of the data for certain days during these periods (table 17) indicate very clearly the extent of these increases and decreases.

TABLE 17

Family 58. Table showing distribution of the spine numbers of progeny of high and low lines on three dates. On Dec. 31, the distribution was almost "normal" for the two lines. On Jan. 15 the spine number had increased in both, but more noticeably in the low line. On Jan. 18 a reduction in the number of spines in both lines to near the normal is indicated; and on Jan. 26, an increase again in both lines.

Date	Line	Spine number							Total number of progeny	Mean spine number
		8	9	10	11	12	13	14		
Dec. 31	High			1	13	17	5		36	11.72
	Low	3	2	18	8	5			36	10.27
Jan. 15	High			1	4	5	4		14	11.85
	Low				4	5	1		10	11.70
Jan. 18	High			2	3	7			12	11.41
	Low			4	4	3			11	10.91
Jan. 26	High				3	4	3	1	11	12.18
	Low			1	3	1	1		6	11.66

Divergences similar to those obtained in family 58 are no doubt occurring in nature at all times, and probably lead to greater differences there than those recorded above. Such differences as exhibited by families 80 and 83 (see figures 9 and 10) might have arisen in this way, and, while the means of certain branches from these lines might approach each other and even coincide, the lines as a whole would remain distinctly different, each possessing a different mean. (See figure 27.)

Selection within the high line

The problem that was next undertaken was to determine whether high and low lines could be obtained from each of the high and low lines al-

ready secured. Accordingly a few specimens were selected from the high line as progenitors of a new high line, and a few were selected from the high line as progenitors of a new low line. These selections were made, as formerly, on the basis of past performance, and all parents and progeny were kept during a period of six days. The results as in-

TABLE 18

Family 58. High line. Results of selection for high and low numbers of spines (Feb. 8 to Feb. 13, 1918).

Period	Number of progeny	High branch		Low branch		Difference
		Number of progeny	Mean spine number	Number of progeny	Mean spine number	
1 (6 days)	595	300	11.47	295	11.48	-.01

licated in the diagram on page 110 and in table 18 show that the mean spine number of the 300 specimens in the high line was 11.47 and that of the 295 specimens in the low line was 11.48—a difference of only .01. This slight result and lack of time necessitated the discontinuance of this experiment so that selection could be continued in the low line, but the data obtained from the latter leave no doubt that further work would have been successful.

Selection within the low line

Specimens with a high spine number and those with a low spine number were selected on the basis of past performance from those of the low line that were living at the end of the non-selection periods. These were reared during three selection periods of 4, 9 and 6 days respectively. Selection was practiced at the end of each period and also during the periods. As shown in the diagram on page 110 and in table 19 a divergence was obtained of .19, .37, and .34 respectively during the three selection periods. A non-selection period was then inaugurated lasting for 11 days (table 20). At the beginning of this period a representative group of specimens was selected in each line. A divergence of .44 resulted. Since the data thus obtained seemed to prove that two lines differing in their heritable characteristics had been obtained from the low line, the work with these lines was discontinued.

It seems probable also that divergences of a similar sort would be

TABLE 19

Family 58. Low line. Results of selection for high and low numbers of spines (Feb. 8 to Mar. 2, 1918).

Period	Number of progeny	High branch		Low branch		Difference
		Number of progeny	Mean spine number	Number of progeny	Mean spine number	
1 (4 days)	207	71	11.57	136	11.38	.19
2 (11 days)	228	103	11.29	125	10.92	.37
3 (8 days)	287	196	11.21	91	10.87	.34
23 days	722	Mean difference				.30

TABLE 20

Family 58. Low line. Spine numbers during non-selection period (Mar. 2 to Mar. 13, 1918).

Period	Number of progeny	High branch		Low branch		Difference
		Number of progeny	Mean spine number	Number of progeny	Mean spine number	
1 (11 days)	224	125	11.38	99	10.94	.44

obtained if these two divergent branches of the low line were subjected to further selection as described above.

Thus a family containing all of the descendants of a single individual reproducing vegetatively would probably be found to consist of a large number of subfamilies, each differing slightly from the others in heritable characteristics. These divergent subfamilies would correspond in heritable characteristics to the small families derived from many "wild" individuals such as those described in part 3 of this paper (p. 105).

PERMANENT DIVERSITIES IN DIAMETER OF SHELL AMONG THE DESCENDANTS OF A SINGLE SPECIMEN PRODUCED BY VEGETATIVE REPRODUCTION

a. Diversities in diameter of shell between the high and low lines of family 58

The question with which we are concerned here is, Do permanent diversities in measurable heritable characteristics other than spine number

appear among the descendants of a single specimen reproducing vegetatively?

It was impossible to measure all of the specimens of family 58 that were examined for spine number, but at intervals during the progress of the work measurements were made of enough of the specimens to give significant means. The data obtained are presented in tables 21 to 24 and may be summarized as follows:

TABLE 21

Family 58. Table showing distribution of diameters of shells within the high and low lines during the non-selection period (Jan. 15 to Jan. 26, 1918.) The unit of measurement is 4.3 microns.

Line	Diameters							Total number of progeny	Mean diameter
	24	25	26	27	28	29	30		
High	1	5	25	37	48	10	1	127	27.26
Low	2	10	25	65	28	6		136	26.92

Difference, .34

Measurements were made of all progeny in both high and low lines from Jan. 15 to Jan. 26, 1918, a period covering parts of the second, third and fourth non-selection periods. Table 21 gives the distribution of the diameters; the total number of progeny and the means obtained. The mean diameter of shell in the high line was greater than that of the low line. The difference of .34 unit is not great, but is as large as could be expected considering the fact that the mean difference in spine number at the same time was only .41. As was to be expected from previous tabulations a high correlation was found to exist between diameter of

TABLE 22

Family 58. Correlation table for parents and offspring with respect to diameter of the shell in both high and low lines during the non-selection period (Jan. 15 to Jan. 26, 1918). The unit of measurement is 4.3 microns. Correlation, .489 \pm .035.

		Parents						
		24	25	26	27	28	29	30
Progeny	24		2	1				3
	25		1	8	3	2		14
	26		1	5	10	24	4	44
	27			4	13	45	23	88
	28			1	5	17	31	63
	29					1	3	5
	30							
		1	13	37	90	63	12	217

shell and spine number (table 22). We may conclude, therefore, that permanent diversities in the diameter of the shell, as well as in spine number, have appeared among the descendants of specimen 58 during vegetative reproduction. It is remarkable how constant these characteristics are among the progeny of a single specimen, but this very constancy emphasizes the importance of the differences observed.

b. Diversities in diameter of shell between the high and low branches of the low line of family 58

The next question is, Is there a difference in the diameter of the shell in the high and low branches of the low line of family 58 corresponding to the difference in spine number? Measurements were made of all progeny in these two branches from Feb. 14 to Feb. 23 and from Mar. 7 to Mar. 13, 1918. The distribution of diameters of shells, the total number of progeny, and the means for both these periods and for the two periods combined are tabulated in table 23. This table shows that

TABLE 23

Family 58. Low Line. Table showing the distribution of diameters of shells within the high and low branches of the low line for two periods (Feb. 14 to Feb. 23, and Mar. 7 to Mar. 13, 1918). The unit of measurement is 4.3 microns.

Branch	Date	Diameters						Total number of progeny	Mean diameter
		25	26	27	28	29	30		
High	Feb. 14-23	4	12	42	25	8		91	27.23
Low	Feb. 14-23	1	27	56	34	6		124	27.13
High	Mar. 7-13	2	18	38	29	5	1	93	27.21
Low	Mar. 7-13	8	28	20	15	5		76	26.68
High	Feb. 14-23 Mar. 7-13	6	30	80	54	13	1	184	27.22
Low	Feb. 14-23 Mar. 7-13	9	55	76	49	11		200	26.96

Difference .26

during the period from Feb. 14 to Feb. 23, the high branch had a mean shell diameter .10 units greater than that of the low branch; that during the period from Mar. 7 to Mar. 13 this difference was .53 units; and that when the two periods are combined the difference is .26 units. At the same time the mean difference in spine number was .40.

These data prove that there is a difference between the mean diameter of the shells in the high and low branches of the low line of family 58 corresponding to the difference between them in mean spine number. The positive correlation between these characters within this family is similar to that found in a "wild" population (compare tables 24 and 1).

TABLE 24

Family 58. Correlation table for spine number and diameter of the shell in both high and low lines during the non-selection period (Jan. 13 to Jan. 26, 1918). The unit of measurement is 4.3 microns. Correlation, $.255 \pm .042$.

		Diameter							
		24	25	26	27	28	29	30	
Spine number	9			2	2				4
	10	I	2	13	20	14			50
	11		7	16	36	24	5		88
	12		4	5	27	22	4		62
	13			I	3	7	3	I	15
	14				2	I			3
		I	13	37	90	68	12	I	222

DIVERSITIES IN DIAMETER OF SHELL AND IN SPINE NUMBER DUE TO SUDDEN LARGE VARIATIONS ("MUTATIONS")

As stated above, the constancy in both diameter of shell and spine number during the vegetative reproduction of *Arcella dentata* is remarkable. The difference in diameter of shell between parent and offspring is seldom greater than 2 units (each unit being 4.3 microns) and that in spine number usually not more than 1 or 2. In four cases there appeared in the cultures under observation, differences in diameter of shell between parent and offspring that were so great as to be conspicuous. In all of these cases the parents belonged to the low line (**E**) and the offspring were smaller than their parents. Three of the branches established by these small offspring can be disposed of very briefly; the other two deserve more extended treatment.

Branch **EF**

Specimen **EF** was the ninth offspring of a parent (58.I.I.I.4.2.I.I.I.I.I.I.I.I) that had nine spines and measured 25 units in diameter. **EF** also had nine spines but measured only 22 units in diameter. At the time **EF** appeared (December 22) the mean spine number of the low line (**E**) was 10.48 and the mean diameter 26.81 units. Part of the descendants of **EF** are shown in pedigree form in figure 18. The diameter of shell and spine number are small in the first generation, but increase to

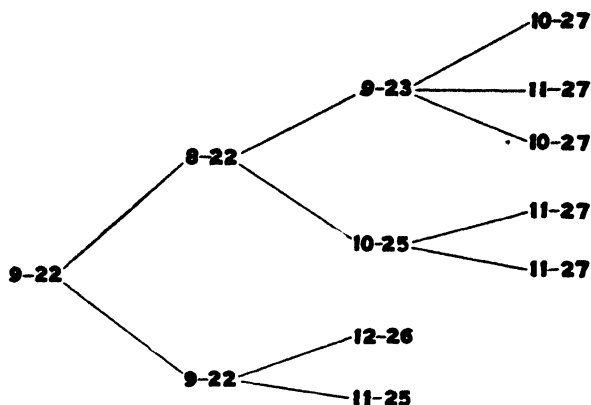


FIGURE 18.—Part of the pedigree of branch EF. The number before each dash is the spine number; that after each dash, the diameter in units of 4.3 microns.

the mean of the line in the third generation. The small diameter of EF was therefore only temporary and probably due to some environmental influence, and the small diameters of the first and second generations indicate that when the decrease in size is not due to a change in the germ plasm, it requires about three generations for the descendants of a very small specimen to reach the normal size.

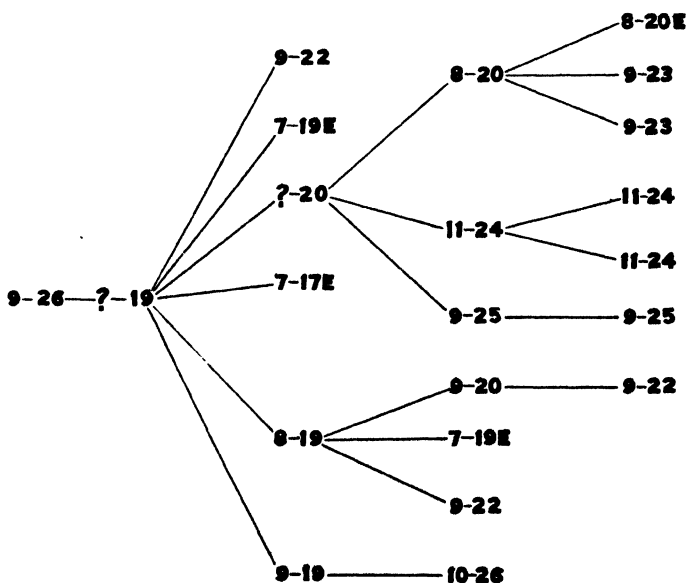


FIGURE 19.—Part of the pedigree of branch E421. The number before each dash is the spine number; that after each dash, the diameter in units of 4.3 microns.

Branch E421

The progenitor of this branch, whose full label is 58.I.I.I.4.2.I.I.I.I.4.2.I., arose on December 8 from a specimen with nine spines and a diameter near the average of the parent line. Figure 19 gives part of the pedigree of this branch and shows that although small specimens still appeared in the fourth generation, there was a gradual increase in diameter and spine number that would probably have resulted in the attainment of a mean spine number of about 10.69 and a mean diameter of shell of about 26.81 which were those of the line **E** (on December 27, 1917), from which this branch was derived. It is, of course, possible that some of the sub-branches may have remained small, but lack of time necessitated the elimination of the entire branch.

Branch EM

Specimen **EM** was also a member of branch **E** of the low line. Its entire label is 58.I.I.I.4.2.I.I.I.I.I.I.I.I.I.3. It was regular in form, had 8 spines, and was only 18 units in diameter (figure 22, **B**); whereas the mean spine number of the low line at the time **EM** appeared (December 13) was 10.69 and the diameter (on December 27) was 26.81

TABLE 25

Family 58. Branch EM. Table showing the number of progeny and their mean diameter for ten generations (Dec. 13, 1917, to Jan. 19, 1918). The unit of measurement is 4.3 microns.

Number of generation	Number of progeny	Mean diameter
1	10	23.40
2	32	25.34
3	54	26.01
4	52	26.34
5	47	26.31
6	41	26.41
7	27	27.11
8	18	27.00
9	24	26.62
10	98	26.51
Total	403	

TABLE 26

Family 58. Branch EM. Correlation table for spine number and diameter of shell of 97 specimens. The unit of measurement is 4.3 microns. Correlation $.368 \pm .059$.

	Spine number					
	8	9	10	11	12	
Diameter						
23		2	1			3
24		1	3	2		6
25	1	1	14	6	1	23
26	1	2	23	19	6	51
27			3	8	1	12
28				1	1	2
	2	6	44	36	9	97

units. The parent of **EM** had 10 spines and was 27 units in diameter. This parent gave rise to offspring normal in diameter and spine number both before and after **EM** appeared. Measurements were not made of the specimens but the spine number is indicated in the pedigree shown in figure 20.

By December 29, the descendants of **EM** had increased to 58 and it became necessary to eliminate some of them. Since the diameter and spine number seemed to increase at each succeeding generation, it was decided to try to get enough specimens in each of the first ten generations to furnish significant means for each generation. As tables 25, 26 and 27 show, **EM** produced ten offspring whose mean diameter was 23.40. There was a gradual increase in the mean diameter during the first four generations (figure 21) and then fluctuations until the tenth generation (January 19, 1918) when the work was stopped. During the period when these progeny were obtained (December 13, 1917, to

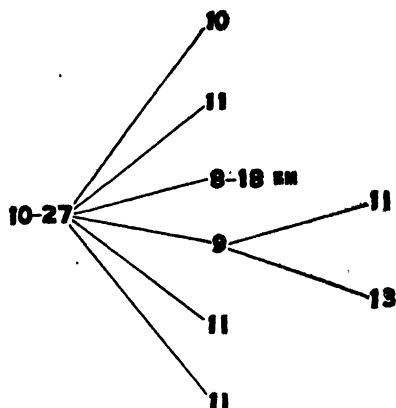


FIGURE 20.—Pedigree showing the parent of specimen **EM** and five of the sisters of **EM**. The number before each dash is the spine number; that after each dash, the diameter in units of 4.3 microns.

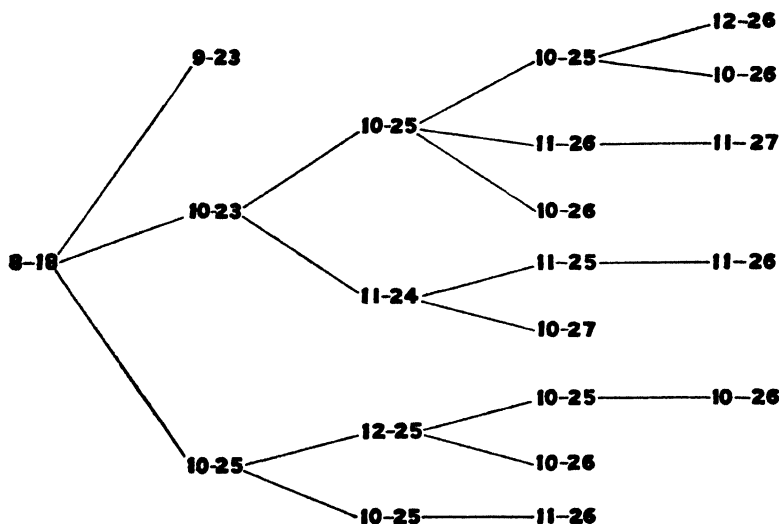


FIGURE 21.—The first part of the pedigree of branch EM. The number before each dash is the spine number; that after each dash, the diameter in units of 4.3 microns.

January 19, 1918) the mean diameter of the low line **E** from which **EM** arose was 26.81 units (on December 27, 1917) and the mean spine number 10.62. These data indicate that the divergence of **EM** and the first 4 or 5 generations derived from it was only temporary and that **EM** could not be called a mutation in the sense that its germ plasm had become permanently modified. As noted above in the case of branch **EF** (page 128) when for some reason a specimen appears that is markedly different in size from its parent, this decrease in size being due to some environmental factor, it seems to require several generations before the parental condition is regained. At certain times during the cultivation of branch **EM** sudden increases and decreases in the diameter and spine numbers of all the progeny occurred similar to those noted in the other

TABLE 27

Family 58. Branch EM. Table showing a notable increase and subsequent decrease in spine number and in diameter of the shell. The unit of measurement is 4.3 microns.

Date	Number of progeny	Mean spine number	Mean diameter
Feb. 2	9	11.22	27.55
Feb. 7	7	12.28	28.85
Feb. 9	8	10.87	27.50

branches of family 58 (see page 121). Table 27 gives the data for a few days during which a notable increase and subsequent decrease took place.

Branch ED

The work carried on with this branch was more extensive than with the other branches described above, and led to the greatest divergences that were discovered during the entire work. The specimen **ED** appeared on December 22, 1917. Its complete label is 58.1.1.1.4.2.1.1.1.1.1.1.1.8. It was the eighth offspring of a specimen that was of average size and whose other offspring were of average size. **ED** itself is very close to the mean of the race, measuring 26 units in diameter and possessing 9 spines. The line from which it arose had a mean spine number at this time of 10.48 and a mean diameter of 26.81 units. **ED**

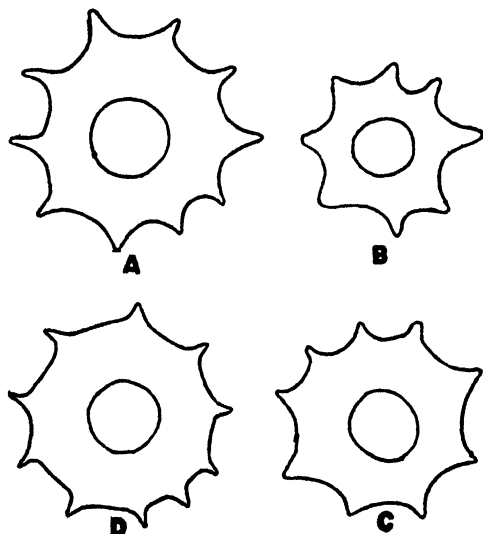


FIGURE 22.—Members of the branch EM. A, the parent of EM with 10 spines and a diameter of 116 microns. B, EM with 8 spines and a diameter of 77 microns. C, the first offspring of EM, with 9 spines and a diameter of 99 microns. D, a typical member of the fourth generation with 10 spines and a diameter of 116 microns. $\times 207$.

produced 8 offspring with a mean diameter of 20 units and a mean spine number of 8.80. The diagram on page 133 (figure 24) gives a portion of the pedigree of this branch; figure 23 on the same page indicates graphically the principal phases of the work; and table 28 gives the mean diameters and spine numbers of the generations from 1 to 24.

All parents and offspring in this branch were kept for a period of 20

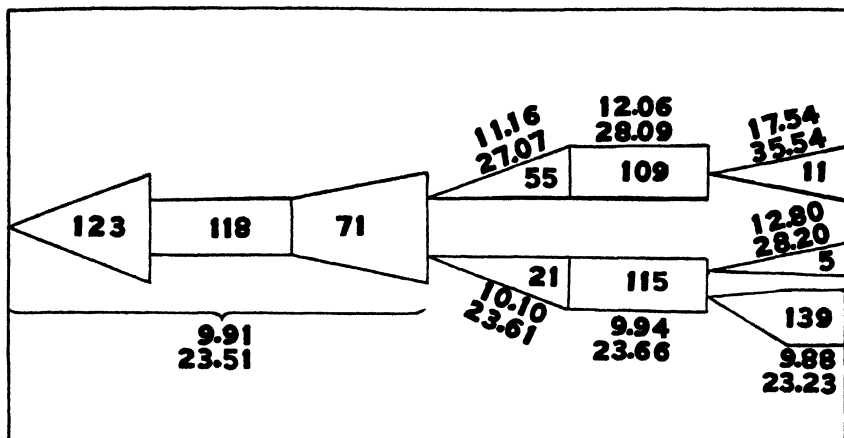


FIGURE 23.—Diagram showing data obtained during the cultivation of branch ED. The data are arranged as in figure 11.

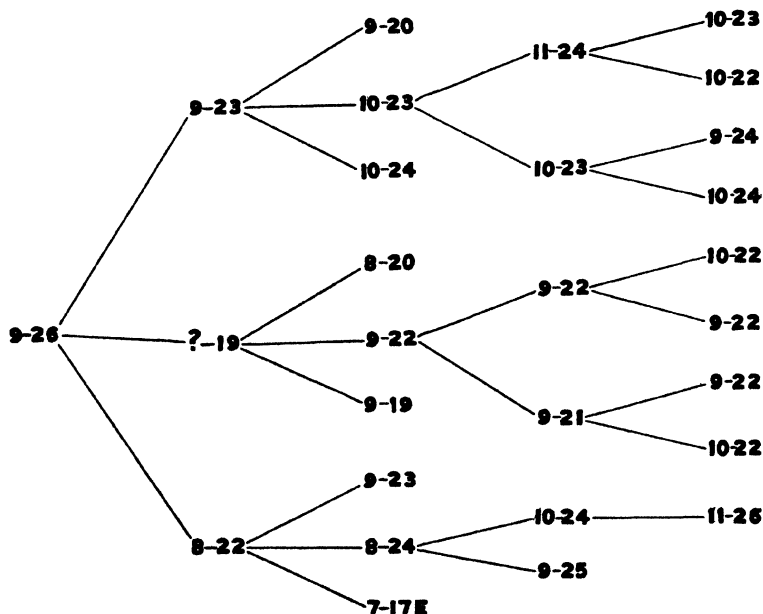


FIGURE 24.—Part of the pedigree of branch ED beginning with specimen ED. The number before each dash is the spine number; that after each dash, the diameter in units of 4.3 microns. The letter E indicates that the shell was empty.

days until there were 123 in all. Then a representative lot of 22 specimens were taken and for a period of 27 days all parents were eliminated

TABLE 28

Family 58. Branch ED. Table showing the mean diameter and mean spine number for generations 1 to 24 (Dec. 22, 1917 to Feb. 22, 1918). This table includes the large specimens belonging to the subbranch EDA, but not the members of the subbranches EDB and EDC. The unit of measurement is 4.3 microns.

Number of generations	Number of specimens	Mean diameter	Mean number of spines
1	8	20.00	8.80
2	12	21.75	9.25
3	28	22.78	9.74
4	28	23.57	10.10
5	20	24.30	10.00
6	22	24.13	10.54
7	15	23.60	9.66
8	10	23.50	10.10
9	9	23.88	10.22
10	13	24.00	10.30
11	9	23.44	10.22
12	8	23.12	9.37
13	6	23.83	9.33
14	6	23.33	9.66
15	14	23.57	9.57
16	24	23.50	10.08
17	19	23.42	9.84
18	6	24.33	9.33
19	15	24.53	10.33
20	15	24.00	10.06
21	11	23.73	10.00
22	8	23.37	10.25
23	5	24.00	10.40
24	1	24.00	9.00
1-24	312	23.51	9.91

as soon as they had reproduced and the offspring were kept. Then for 11 days both parents and offspring were kept. During these three periods, totaling 58 days, records were made of 312 offspring. These had a mean diameter of 23.51 units and a mean spine number of 9.91. During the same period the low line **E** from which **ED** arose had a mean diameter of 27.05 and a mean spine number of 10.99. There is thus a difference in diameter of 3.54 units and in spine number of 1.08.

In the fifth and sixth generations of this branch there appeared in one subbranch a remarkable increase in diameter and in spine number, as shown in the diagram on p. 137 (figure 26, **EDA**). This increase may be tabulated as follows:

Generation	Spine number	Diameter
1	8	22
2	8	24
3	10	24
4	11	26
5	13	31
6	16	32

Several empty shells were thrown by members of this subbranch, but empty shells were of rather frequent occurrence in all the lines studied and in the other lines did not cause any such remarkable changes. There were only three very large specimens produced; these measured respectively 31, 31, and 32 units in diameter, and possessed 13, 14 and 16 spines respectively. No further offspring were obtained from these nor from their progenitors although every care was taken to keep them in favorable cultural conditions.

On February 20 it was decided to try selection with those members of the branch **ED** that remained alive at that time. Four specimens were selected on the basis of past performance to start a high line and 4 to start a low line. The diameters and spine numbers of these were as follows:

High line		Low line	
Diameter	Spine number	Diameter	Spine number
24	10	22	9
25	11	22	10
25	10	22	9
26	12	19	9

During a period of 9 days fifty-five offspring were produced in the high line with a mean diameter of 27.07 units and a mean spine number of 11.16, and 21 offspring in the low line with a mean diameter of 23.61 and a mean spine number of 10.10 (table 29).

Twenty-two representative specimens were then taken from the high line and all of the 21 in the low line were kept. All parents were eliminated as soon as they reproduced and the offspring retained. The number of offspring and means obtained during this period of 13 days are given in table 29.

Four specimens were then selected from the high line for the purpose

TABLE 29

Family 58. Branch ED. Table showing mean diameter and mean spine number during one period (Feb. 20 to Feb. 28, 1918) and a subsequent non-selection period (Mar. 1 to Mar. 13, 1918). The unit of measurement is 4.3 microns.

Period	Number of progeny	High branch			Low branch			Mean difference	
		Number of progeny	Mean spine number	Mean diameter	Number of progeny	Mean spine number	Mean diameter	Spine number	Diameter
Selection (9 days)	76	55	11.16	27.07	21	10.10	23.61	1.06	3.64
Non-selection (13 days)	224	109	12.06	28.09	115	9.94	23.66	2.12	4.43

300

of determining how large a line could be obtained. These four gave rise to 11 offspring and then stopped reproducing (figure 26, **EDB**). No more offspring appeared, although the specimens were very carefully looked after. These 11 offspring had a mean diameter of 35.54 units and a mean spine number of 17.54. The largest specimen measured 40 units in diameter and possessed 20 spines. This is the largest specimen discovered during the entire course of the work. Arcellas with from 13 to 15 spines and with a diameter of from 34 to 36 units were taken from the pond and many of them reared in the laboratory in other lines, and

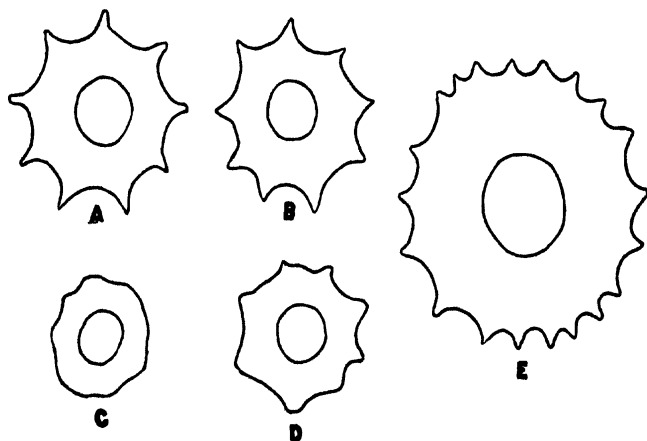


FIGURE 25.—Members of the branch ED. A, the progenitor of the branch ED with 9 spines and a diameter of 112 microns. B, the first offspring of ED, with 9 spines and a diameter of 99 microns. C, the second offspring of ED, with indistinct spines and a diameter of 82 microns. D, the third offspring of ED, with 8 spines and a diameter of 95 microns. E, the largest specimen from the subbranch EDB, with 20 spines and a diameter of 172 microns. $\times 207$.

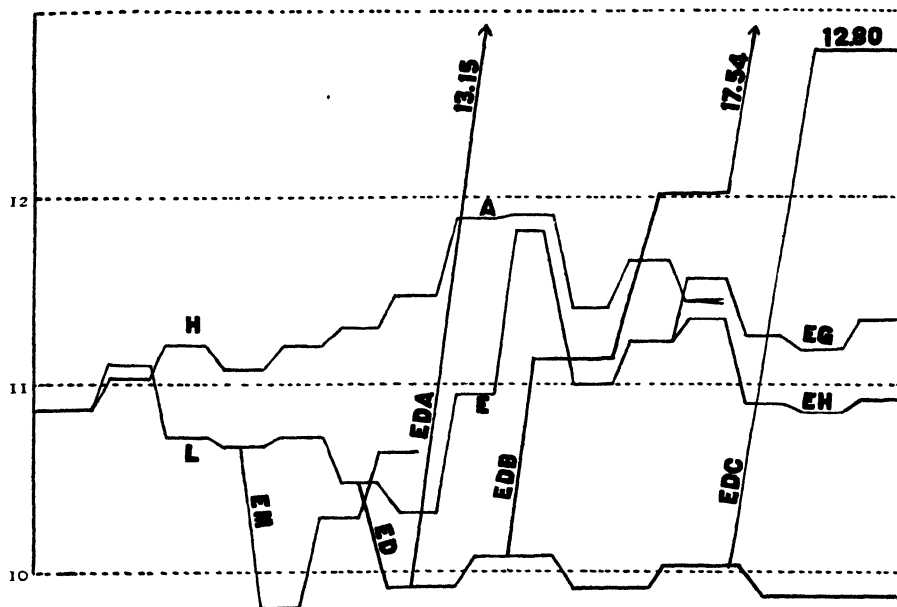


FIGURE 26.—Diagram showing the magnitude of the diversities in spine number that appeared during the vegetative reproduction of family 58. The distances between the horizontal lines indicate the differences between the mean spine numbers of the lines studied. A mean difference of one spine is equal to a vertical distance of 37 mm in the diagram. The diagram shows that before selection was begun, the mean spine number of the members of the family was 10.87. A high line (H) and a low line (L) were obtained during six selection periods with a final difference of 11.48 — 10.32, or 1.16. The high and low lines remained different during four succeeding non-selection periods, the final difference being 11.69 — 11.26, or .43. Selection was then started in the high line and was continued for one period resulting in a difference of only .01 between the high branch AG and the low branch AH. Selection was also begun in the low line at the same time and was carried on for three periods, EG and EH, a final difference of 11.21 — 10.87, or .34, being obtained. This was followed by a single non-selection period which ended with a difference of 11.38 — 10.94, or .44.

During the third selection period a very small individual, EM, appeared in branch E, but this proved to be only a temporary divergence since its descendants by the end of three periods had attained a mean spine number (10.65) at least equal to that of the parent branch E.

During the fifth selection period an individual, named ED, appeared in branch E and produced descendants with a much lower spine number than the parent branch. The main branch ED remained at a lower level than the parent branch E, but three branches, EDA, EDB and EDC originated from it which reached a higher mean spine number than any other lines studied, namely, 13.15 in branch EDA, 17.54 in branch EDB, and 12.80 in branch EDC. More detailed data will be found in diagram 11 on page 110, and several other branches that were studied will be found described in the text, having been omitted from the diagram for the sake of simplicity.

even specimens with 16 or 17 spines and a diameter of 37 units were recorded, but none were found that approached this in number of spines or in diameter except in this particular branch of the line **ED**.

At the same time that the high line in this branch **ED** was being studied, an attempt was made to obtain a further divergence between the specimens in the low line. Two specimens were selected for the high line and two for the low line as follows:

High line		Low line	
Spine number	Diameter	Spine number	Diameter
10	27	10	24
12	27	9	22

Only five offspring were produced by the two specimens selected for the high line (figure 26, **EDC**). These had a mean diameter of 28.20 units and a mean spine number of 12.80. No further offspring appeared and all of the specimens died.

In the low line after the 2 selected specimens had increased to 22, parents were eliminated and offspring kept whenever reproduction occurred. This procedure was maintained for 42 days (from March 14 to April 24, 1918). Table 30 shows the correlation between diameters and spine

TABLE 30

Family 58. Branch ED. Correlation table for spine number and diameter of shell in low subbranch of the low branch (March 14 to April 24, 1918). The unit of measurement is 4.3 microns. Correlation, .531 \pm .041.

	Spine number						
	7	8	9	10	11	12	
Diameter							
16	1						1
17							0
18							0
19							0
20			2				2
21			2				2
22	1	1	11	5	1		19
23		1	18	31	9		59
24		1	8	21	13	3	46
25			1		7		8
26					2		2
	1	3	42	57	32	3	139

numbers during this period. The mean spine number of the 139 progeny recorded is 9.88 and the mean diameter of the same progeny is 23.23

(see figure 23). All progeny were below the mean diameter of the line from which their original progenitor was derived and the mean diameter of this branch is nearly the same as the mean of the first large group of 312 reared before selection was begun (figure 23).

It seems clear, therefore, that the line **ED** had become permanently different from the original line **E** from which it arose both with respect to diameter and to spine number. It seems strange that this small derivative of the original line **E** should give rise to three branches **EDA**, **EDB** and **EDC** which are larger than any other branches in the entire family 58 (figure 26). Another phenomenon that needs to be accounted for is the dying out of all of the members of these large branches. The probable reason for this will be pointed out in a later paper, where the relations between nucleus and cytoplasm are described.

Some time after branch **ED** appeared it was discovered in another line that specimens with a single nucleus could be obtained by removing one of the two normally present, and that these uninucleate specimens would produce uninucleate progeny. These uninucleate progeny in every case had shells that were smaller than those of their modified parents as well as fewer spines. All uninucleate progeny that appeared later likewise possessed smaller shells. This discovery led to an examination of the nuclear condition of the members of branch **ED** since the small size of these specimens might be due to the presence of only one nucleus instead of the normal number, two. It was impossible to determine with certainty the nuclear condition of all of the progeny of **ED** because of their small size, the color of the shell, and the large amount of food material often contained in them, but in a large number of cases two nuclei were plainly visible even in some of the smallest specimens measuring 22 units in diameter or less, and only in three or four specimens was it possible to conclude that only one nucleus was present. If the few cases of this kind were thrown out of the foregoing calculations, they would make practically no difference in the results.

EMPTY SHELLS

At irregular intervals during the course of this investigation new shells were formed which separated from the parent organism without being provided with any protoplasm. These empty shells appeared in all the lines under cultivation and no factors were discovered that would account for their production. In family 58 a total of 59 empty shells was recorded or about one percent of the specimens studied in this entire family. Since the empty shells were almost always smaller than their

TABLE 31

Family 58. Table showing the relations between empty shells and their parents and older and younger sisters, with respect to spine number and diameter in ten cases. The unit of measurement is 4.3 microns.

Parent		Empty shell			Older sister		Younger sister	
Spine number	Diameter	Progeny number	Spine number	Diameter	Spine number	Diameter	Spine number	Diameter
11	27	4	8	22	10	25	9	26
12	29	2	9	20	12	28	11	26
10	24	2	8	19	11	24	12	27
9	20	2	7	18	10	22	9	22
9	24	2	8	19	10	25	9	19
11	26	2	10	20	12	27	12	26
11	26	8	8	22	13	27	11	27
8	23	2	9	21	10	24	9	23
8	19	2	7	19	9	20	9	22
9	22	3	7	20	9	22	7	21

parents and possessed a lesser number of spines, they were not included in the data presented in the preceding pages.

Table 31 gives the data with regard to ten representative empty shells. In a few cases the number of spines possessed by the empty shell could not be determined; in a number of cases the diameters of the empty shells, parents, and sisters were not obtained; and in 35 instances the empty shell represented the first offspring and hence had no older sister, and some of the parents were eliminated before a younger sister was produced.

The data obtained show that of the 59 empty shells in family 58,

35 were the first offspring of their parents;

12 were the second;

4 were the third;

3 were the fourth;

3 were the fifth; and

2 were the eighth.

The comparatively large number belonging to the first and second generations is no doubt due to the fact that most of the specimens in the family were eliminated after producing one or 2 offspring. The empty shells possessed fewer spines than their parents in 45 cases, a greater number in 5 cases, and an equal number in 2 cases. They were smaller than their parents in 38 cases; larger in 2 cases, and equal in size in 2 cases.

The relations between the empty shells and their older and younger sisters, i.e., the progeny of the same parent just preceding or succeeding them, were determined with the following results. The spine number of the older sister was greater than that of the empty shell in 18 cases, less in 1 case, and equal to it in 1 case. The spine number of the younger sister was greater than that of the empty shell in 47 cases, less in 2 cases, and equal to it in 5 cases. The spine number of the older sister was greater than that of the younger sister in 6 cases, less in 5 cases, and equal to it in 2 cases. The spine number of the parent was greater than that of the younger sister in 16 cases, less in 21 cases, and equal to it in 5 cases. It seems evident from these results that the production of empty shells has no appreciable influence upon the spine number of later offspring (younger sisters) from the same parent.

Similar relations have been found with regard to the size of the empty shells, their parents, and older and younger sisters. These relations are as follows:

Empty shells were smaller than the parents in 38 cases;

larger in 2 cases;

equal in 3 cases.

Empty shells were smaller than the older sisters in 16 cases;

equal in 1 case.

Empty shells were smaller than the younger sisters in 36 cases;

equal in 2 cases.

Younger sisters were larger than the older sisters in 5 cases;

smaller in 6 cases;

equal in 2 cases.

Younger sisters were larger than parents in 19 cases;

smaller in 12 cases;

equal in 5 cases.

These data show that the empty shells are almost always smaller than their parents, and smaller than their older and younger sisters. The influence of the production of the empty shell upon the succeeding offspring (younger sister) from any given parent is indicated in the last set of data presented above. The next offspring produced after an empty shell is formed is in more than half of the cases larger than the parent. Why this should be so is unknown, but the empty shells are doubtless empty because of a disturbance at the time of reproduction and probably there was not sufficient protoplasm for the production of a normal individual. If this were the case there would be a superabundance of protoplasm ready by the time of the next fission and the next offspring

(younger sister) would tend to be larger than its parent. However, there is no appreciable difference in size between the offspring preceding and the one succeeding the production of the empty shells, so until more extensive data are available the causes and effects of empty shell formation will have to remain unexplained.

It is worthy of note, nevertheless, that two of the sudden increases in diameter and spine number in branch **ED** (see figure 26) were both preceded by the formation of empty shells. In a number of other cases, on the other hand, sudden increases in size and spine number occurred in all the lines under cultivation at the same time without the intervention of empty shell production.

In a number of experiments on Arcellas taken from family 58 or directly from the pond, it was found that specimens when deprived of one of their two nuclei always formed an empty shell just before the binucleate condition was regained. Apparently nothing of this kind happened in the 59 cases described above, since in many specimens the nuclear condition was examined and two nuclei were always present (HEGNER 1919).

Conclusion

We may therefore ignore the empty shells in our studies of diversities in these organisms, at least until we know more about the conditions that cause their formation.

DISCUSSION

A number of questions have suggested themselves during the course of these studies which may profitably be grouped together in this discussion.

1. How does selection operate when practiced on Arcella during vegetative reproduction?

This question is of considerable importance since the significance of the results recorded in this contribution can not be realized until the fundamental difference between selection among sexually and asexually reproducing organisms is clearly understood. If it had been possible to take care of all of the descendants resulting from the vegetative reproduction of the single individual (No. 58) with which these lines were started, and if the pedigree of all these descendants were constructed according to the scheme indicated on page 110, the same lines and branches would appear in this pedigree that have been described in the foregoing pages, but besides this hundreds of other branches would also appear,

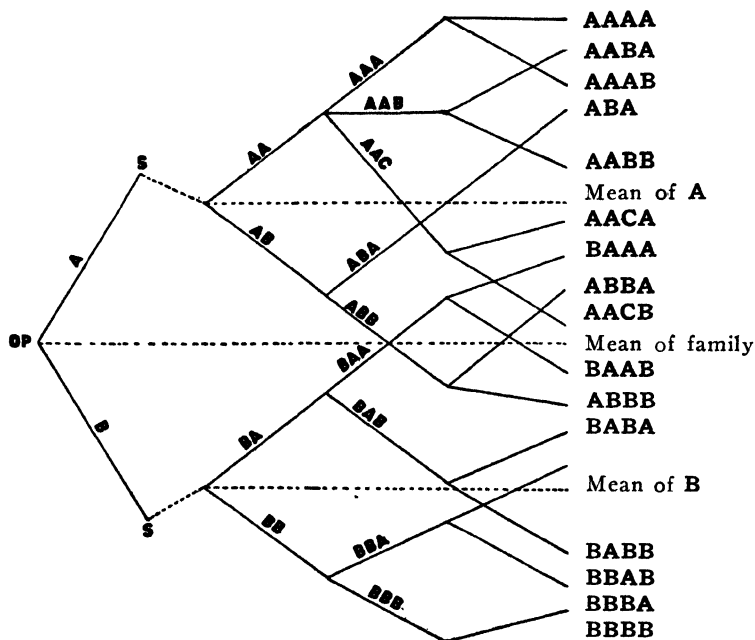


FIGURE 27.—Diagram showing the various heritably diverse branches with respect to spine number and diameter that may arise from a single specimen of *Arcella dentata* during vegetative reproduction. OP = original progenitor. A high line A and a low line B are isolated by selection. The means of these lines then regress slightly toward the mean of the entire family, but soon become almost stationary although branches from both lines cross each other. For further explanation, see page 145.

such as are indicated in figure 27, branches that were eliminated during the experiments because of lack of time and strength to keep them going. In other words, there has been no selection of existing heritable factors that are contributed to the progeny by two different parents, as is usually the case when selection is practiced among sexually reproducing organisms; but specimens have been picked out from a pedigree resulting from the successive divisions of a single germ plasm.

2. Are shell characteristics influenced by the environment?

There is a peculiarity exhibited by many of the correlation tables that have been made up that needs consideration. Offspring of parents with a spine number near the lower limit, e.g., those of parents with 8 or 9 spines, possess an exceptionally high mean spine number.

Two sets of factors must be considered in interpreting such tables, (1) hereditary factors and (2) environmental factors. It seems perfectly clear that the influence of heredity is very strong. As regards the

environment, although this was kept as uniform as possible at all times, still small solid objects such as minute grains of sand might modify the shell of the offspring while it is still in a plastic condition, so as to prevent the development of the complete (hereditary) spine number. This would account for *some* of the offspring with 7, 8 and 9 spines that were produced by parents with 11 and 12 spines. The hereditary factor would tend to express itself again in the next generation, hence, e.g., offspring with 8 spines would produce offspring with 10, 11, 12 or 13, as shown in the tables. This may account for the exceptionally high mean spine number of the progeny of parents with 8 spines as shown in table 5. Many of the low-spined specimens here recorded were not regular, but, as indicated in figure 12, **C**, large spaces sometimes occurred which might be due to the suppression of one or more spines by some obstruction in the environment.

Mechanical environmental factors might thus cause a decrease in spine number, but probably could not produce an increase; thus the mean spine number of the offspring of high-spined parents would tend to be lower than their hereditary constitution. Other environmental factors may affect the organism at the time of reproduction or during the intervals between fissions. In the latter case the results of their influence would not be visible until reproduction occurs, and then only in the offspring, since the shell of the parent is not modified after it is once formed.

In this connection we may also refer to the increases and decreases in spine number that have several times been found to occur simultaneously in all of the lines under cultivation. Examples of this are cited in both the high and low lines of family 58 (page 121) and in branch **EM** (page 131), and data are presented in tables 17 and 27. The most plausible suggestion to account for this is that some cultural condition favorable for the growth of the organisms caused an increase in size, and a subsequent less favorable condition resulted in a reversion to the previous state which we have called "normal." This increase in size would be accompanied by an increase in the spine number, since these two characters are closely correlated.

3. Why was there a decrease in the amount of diversity between the high and low lines of family 58 after selection was discontinued?

The lesser difference between the high and low lines at the end of the non-selection periods than at the beginning is probably due to the appearance in the high line of heritable variations toward a lower spine number, and in the low line of similar variations toward a higher spine num-

ber. Specimens exhibiting such variations would have been removed during the selection periods and hence would not have been allowed to produce offspring which would lower or raise the means in the two lines.

In other words, the differences between the means of the two lines during the selection periods appear greater than they really were, and when selection was stopped, the true condition was revealed. From this time on there should be no great fluctuations in the magnitude of the difference between the means except those due to environmental influences. This proved to be true, as the data show clearly.

Figure 27 was made on the basis of the data presented in this paper to illustrate what probably happens during the vegetative reproduction of *Arcella dentata*. Part of the hereditarily diverse branches that result during the vegetative reproduction of a single specimen are here shown. Suppose for purposes of illustration that we have by selection picked out of the entire pedigree the high line **A** and the low line **B**. If we then discontinued selection we would obtain a pedigree in which lines **A** and **B** would give rise to the branches **AA**, **AB** and **BA**, **BB**, and from these in turn would be derived various other branches, a few of which are indicated in the diagram. Some of these branches would have a mean similar to **A** and **B**, but other branches would have a mean either above or below that of the line from which they sprang. Finally an upper limit, represented in the diagram by branch **AAAA**, would be reached beyond which an increase in size and spine number is not possible, just as there is a well known limit to the size of all species of organisms. And similarly a lower limit. **BBBB**, would also be reached. Some of the branches of the high line **A**, such as **ABBB**, would coincide with or even possess a lower mean size and spine number than certain branches of the low line **B**, such as **BAAB**. And certain branches of the low line **B**, such as **BAAA**, would become higher than some of the branches of the high line **A**, such as **AACB**. The mean size and spine number of the entire group of specimens obtained after selection ceased (**S** in figure 27) would at first decrease slightly in line **A** and increase slightly in line **B**, but thereafter would fluctuate very little and the two lines **A** and **B** would remain distinct indefinitely.

The fact that there appears to be both an upper and a lower limit to the size and spine number in these organisms may account for the slight difference of .07 during the second non-selection period (see figures 11 and 26). When the high line reached a mean spine number of 11.90 during the first non-selection period, its upper limit for this character had about been reached. After this upper limit had been reached by the high

line, the low line continued to increase in spine number until it almost attained this upper limit, having a mean spine number of 11.84 in the second non-selection period. Then when the environment changed and a decline in spine number set in, the mean of the low line decreased more than that of the high line and a difference of .41 resulted during the third non-selection period. A simultaneous increase in spine number again appeared during the fourth non-selection period but at this time there was no near approach to the upper limit and the increase in the high line (.27) was greater than that in the low line (.25).

5. Are diversities due to large or small heritable variations?

The studies of family 58 suggest that the diversities obtained were due to barely distinguishable heritable variations and that an actual shifting of the mean and the mode has occurred in this way. The establishment of branch **ED**, however, as a permanently small line, proves that hereditarily diverse lines may be produced by the appearance of a few extreme specimens. The fact should not be overlooked that when an extreme specimen arises, such as **EM** (page 129), whose diversity is not hereditary, it requires several generations before the mean condition of the line is regained. It may therefore also be true that a large change in the hereditary constitution of a specimen would not appear in its entirety in the first generation, although the f_1 progeny would exhibit it in part, but would become fully manifest only in about the third or fourth generations. While it does not seem probable that the high and low lines of family 58 have originated in this way, still it is a possibility that must be kept in mind.

It also seems probable that mutations may have occurred more often than actually observed, since, because they were not visible in the first generation, they might have been discarded. Especially is this true of mutations toward a lower spine number in the high line and a higher spine number in the low line. This may account for the fact that the supposed mutations discovered in the latter were all smaller than the mean of the line.

6. How do diversities in heritable characteristics originate during vegetative reproduction in *Arcella dentata*, i.e., what is the method of evolution in these organisms?

The germ plasm of organisms that are producing vegetatively is commonly supposed to divide into two qualitatively equal parts at each division and the descendants therefore should all be alike. The fact that branches of such a family as No. 58 are permanently diverse, proves that changes have occurred in the *constitution* of the germ plasm during vegetative reproduction; that is, evolution has taken place. These changes in

the constitution of the germ plasm may be due to two causes, (1) unequal distributions of qualitatively different germinal materials (factors or parts of factors?) during fission, or (2) actual changes (chemical?) in the germ plasm arising spontaneously or initiated by the internal or external environment.

The presence of a conspicuous network of chromidia in *Arcella*, which has been identified as idioplasm, may lead during fission to differences in parent and offspring in chromatin content which would account for the heritable diversities discovered. The removal of part of this chromidial network, however, as will be shown in another paper, has no effect upon the heritable characteristics of the line. It may also be that nuclear reorganization processes occur during vegetative reproduction, such as take place in *Paramecium*, but nothing of the kind has been observed in the thousands of specimens examined, although the nuclei may easily be seen in the living animal. This problem can not profitably be discussed further at this time, since, although more is known about the nuclear phenomena and reproductive processes in *Arcella* than in any other rhizopod, still many of the investigations need to be checked up and there is much still to be learned. *Arcella*, however, offers many advantages for the study of the problem of the method of evolution and further investigations along this line are now in progress.

7. How do the results obtained from these studies of *Arcella dentata* compare with those previously reported by other investigators?

Most of the investigators who have attempted to change the genotype by selection in Protozoa that are reproducing vegetatively, have failed.² MIDDLETON (1915), however, succeeded in obtaining from a single progenitor two lines of *Stylonychia* that differed in fission rate, and JENNINGS (1916) demonstrated the fact that vegetative reproduction in *Diffugia corona* is accompanied by minute heritable variations in a number of measurable characters resulting in branches that differ markedly in their genotypic condition. The investigations reported in this paper show that *Arcella dentata* resembles *Diffugia corona* in this respect and confirm many of JENNINGS's discoveries. Since these two organisms resemble each other very closely, it may be worth while to compare briefly the results of JENNINGS's investigations on *Diffugia* with those reported herein on *Arcella*.

Among the characters of *Diffugia corona* studied by JENNINGS are spine number and diameter. The spines of *Diffugia corona* vary from

² For the literature on this subject see JOHANSEN (1913), MIDDLETON (1915), JENNINGS (1916), ACKERT (1916), ROOT (1918).

0 to 14, those of *Arcella dentata* from 7 to 20. In both organisms, (1) families resulting from the vegetative reproduction of single "wild" specimens were obtained which were hereditarily diverse with regard to spine number; (2) families diverse in shell diameter were discovered; (3) deviations of the parents from the mean spine number and diameter are in part inherited by the offspring; (4) diameter and spine number are closely correlated, and the greater the diameter the more numerous are the spines; and (5) the long-continued selection of the progeny of a single specimen that shows deviations in these characters results in the isolation of lines that are heritably diverse. Mutations occur in both *Diffugia* and *Arcella*, but diversities in lines derived from a single specimen seem to be due rather to the accumulation of many small heritable variations than to large ones.

SUMMARY

1. The main problem attacked in this investigation is, Can heritably diverse lines be recognized among the descendants of a single specimen of *Arcella dentata* produced by vegetative reproduction?

2. *Arcella dentata* was chosen as material because it possesses several definite measurable characters, especially the number of spines and diameter of the shell, which are fully determined at the time of reproduction and are not thereafter affected by growth changes, or by environmental factors (figure 1).

3. "Wild" specimens vary in spine number from 7 to 17 and in diameter from 73 microns to 150 microns; these characters are correlated, and on the average the greater the diameter, the more numerous are the spines. (Tables 1 and 2.)

4. Variations in spine number occur among the descendants of a single specimen produced by vegetative reproduction, and these variations are in part inherited.

5. The hereditary constitution of different families obtained by vegetative reproduction from different "wild" specimens is different with respect to spine number and diameter. A "wild" population therefore consists of a large number of hereditarily diverse families that may be isolated in the laboratory. (Tables 3, 4 and 5.)

6. A single large family (No. 58), containing 5557 specimens and representing 69 generations, was obtained from a single specimen by vegetative reproduction. Selection within this family led to the isolation of hereditarily diverse branches as follows (figures 11 and 26).

(a) Using mostly past performance as a basis for selection, two lines

were obtained during a period of 64 days and involving the study of 1192 specimens, representing 22 generations. The difference in spine number between these two branches at the end of this period was 1.16 and the mean difference for the entire period was .55. A difference in spine number was maintained during a succeeding non-selection period of 35 days; 1325 specimens and 18 generations were studied during this period. The difference in spine number between the high and low lines persisted throughout the non-selection period, but decreased to an average mean of .46. This was to be expected since hereditary diversities are constantly appearing both toward an increase and a decrease in spine number (see page 145). (Tables 6 and 14.)

(b) The low line thus obtained was subjected to a period of selection of 23 days during which 722 specimens were obtained, representing 15 generations. Two hereditarily diverse branches of this low line appeared with a mean difference in spine number of .30 which increased during a short succeeding non-selection period. (Table 19.)

(c) Measurements of the diameters of the low and high lines mentioned in (a) show that these lines also were diverse with respect to the diameter of the shell and that spine number and diameter are correlated throughout the entire family. The mean difference in diameter between the high and low lines was .34 units.³ (Tables 21 and 22.)

(d) Similarly there was a mean difference of .26 units in diameter between the high and low branches of the low lines. (Table 23.)

(e) Four branches of family 58 were studied whose progenitors were markedly smaller and possessed a lesser number of spines than the other specimens in the low line in which they appeared. Three of these branches reverted to the mean condition of the low line within a few generations, but one of them, **ED**, proved to be permanently diverse in both spine number and diameter. It differed from the mean of the low line by 1.08 in spine number and by 3.54 units in diameter. (Figure 23.)

(f) From this branch **ED** there appeared at different times three distinct branches (**EDA**, **EDB**, and **EDC**) containing specimens larger and with more numerous spines than those encountered in any other part of the entire family 58. (Compare figures 13 and 25.)

7. Empty shells are often produced by apparently normal specimens. They are almost always smaller than their parent, and smaller than their older and younger sisters. Their appearance seemed to have no influence upon the heritable diversities studied. (Table 31.)

³ The unit of measurement is 4.3 microns.

8. A large family of *Arcella dentata*, therefore, derived from a single specimen by vegetative reproduction, consists of a number of branches that are hereditarily diverse with respect to diameter and spine number. These diverse branches resemble the hereditarily diverse families that were obtained by vegetative reproduction from different "wild" specimens.

9. The formation of such hereditarily diverse branches appears to be a true case of evolution that has been observed in the laboratory and that occurs in a similar way in nature.

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VARIATION AND SELECTION WITHIN CLONAL LINES OF *LEMNA MINOR*¹

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INTRODUCTION

The experiments herein reported were conducted for the purpose of adding to our knowledge concerning clonal variation and the effect of selection on such variation, in relation to the pure-line concept. It is unnecessary here to include a review of published data. It is, of course, generally recognized that the greater bulk of experimental evidence supports the pure-line theory as elaborated by JOHANNSEN.

MATERIAL USED

The plants used in these experiments are commonly called duckweed

¹ Paper No. 73, Department of plant breeding, CORNELL UNIVERSITY, Ithaca, N. Y.

and belong to the genus *Lemna*. This, according to GRAY (1908), is a genus widely distributed over Europe, Northern Asia and North America, but rare in the Tropics. The duckweeds are small, floating plants without distinct stems or real leaves, and may or may not have roots. They rarely produce flowers, the usual mode of propagation being through budding. The present paper is concerned only with one species, *Lemna minor* Linn.

It is necessary to give a more than passing statement regarding the mode of budding. The main structure of the plant is usually called a frond. Some botanists regard it either as a stem, or leaf, or both fused together. The term "frond" is used throughout this paper. According to BLODGETT (1915) the frond consists of three parts: (a) a terminal leaf, (b) a bud rudiment inclosed by a flattened bud scale and (c) an apical region from which new fronds are developed. Vertical pressure during the early stages of growth causes the splitting of the bud rudiment into two buds which do not develop at the same time. These outgrowths come out as a horizontal series in an overlapping form through the lack of space for vertical succession. The development of the basal region into a stalk or stipe causes the thrusting forward of each new whole structure. In *L. minor* this basal region is attached marginally to the main portion of the frond; in other species, as in *L. polyrrhiza*, it is inserted upon the vertical surface some distance from the edge. Figure 1 shows a parent frond with its offspring still attached to it. The members of the family are numbered consecutively in the order of the time of their appearance.

VARIATION WITHIN A WILD POPULATION

Before studying clonal variations a study within a wild population was made concerning shape and size of fronds, speed of propagation and root habits.

Shape of frond

Figure 2 shows fronds of various shapes taken from a population which was collected on December 10, 1916, from a stagnant creek at the Ithaca fair-grounds. The sketches were made by examining the specimens under Zeiss binoculars and tracing the outlines of the image as thrown over the paper with the aid of a Zeiss camera lucida. In all cases mature fronds, such as had already turned yellow but which were still attached to their offspring were studied, thus eliminating, as far as possible, the effect of different ages. From the figure just referred to it

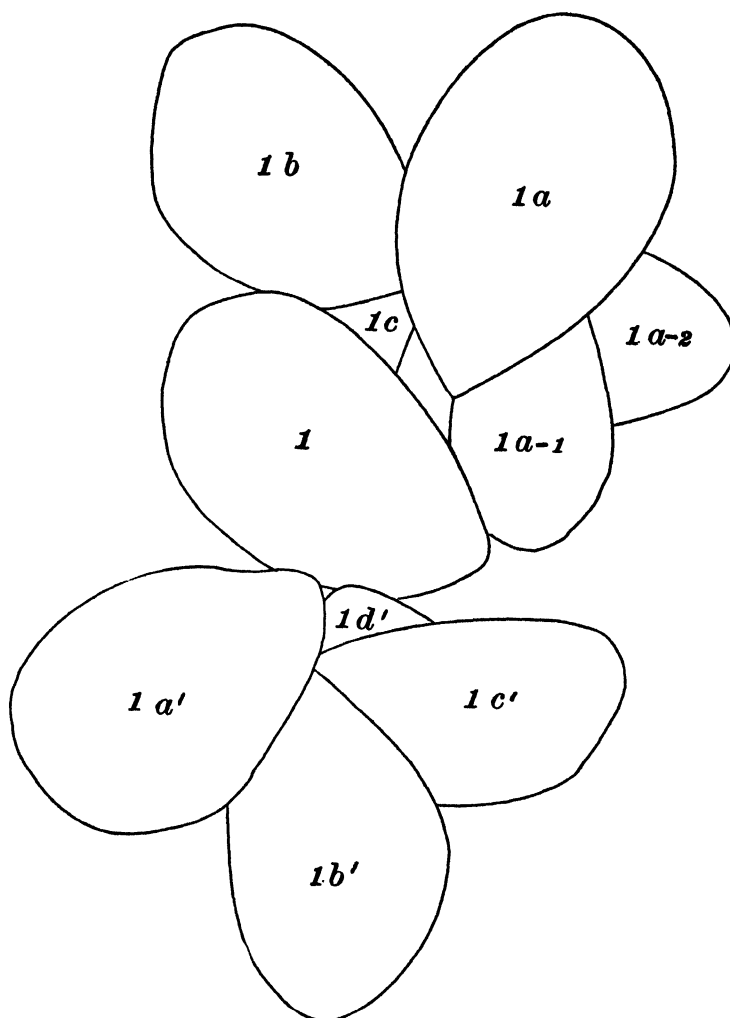


FIGURE 1.—A parent frond with its offspring still attached to it. $\times 16$ diameters.

may be seen that there exist diverse forms of fronds in a wild population. To determine whether or not differences in shape are inherited, that is, to ascertain if different forms represent distinct strains, several fronds were isolated from the wild stock. Each frond was allowed to propagate in a tumbler containing tap water and kept in a greenhouse section in which the temperature was generally 15° C at night and 25° C by day. Preliminary cultural experiments had shown that the plants die after a time if frequent change of water in the culture tumblers

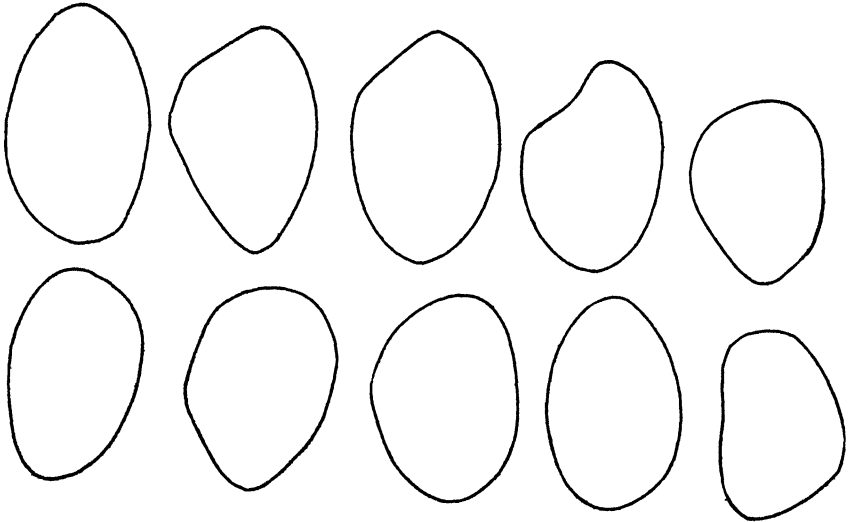


FIGURE 2.—Fronds of *Lemna minor* showing variation in shape within a wild population. $\times 16$ diameters.

is not made. To meet this difficulty, the tumblers were arranged in rows, the members of each row being connected with one another with siphon tubes. By allowing the water to siphon from a big deposit jar into the tumblers at the head of the rows, this water in turn being siphoned into those that follow, a provision was thereby made which permitted a partial but continuous change of water most of the time.

Figure 3 shows camera drawings of fronds from two clones. Each figure shows individuals from one line. From a close study of these and similar unpublished drawings it was seen that while the individuals within a line vary in shape to a greater or less degree, there is much more resemblance among members of the same clone than among those of different lines. It is only fair to conclude from this that in a wild population there exist races of diverse shape.

Speed of budding

The term "speed" does not imply "rate." There is no use of studying variation in rate of budding in *L. minor* since different fronds have the same rate of budding. Each frond produces invariably two buds and no case has yet been reported where more or less than this number has been produced. However, different fronds may require different lengths of time to produce their offspring buds. Speed of budding may be measured either by noting the number of days it takes for a given number

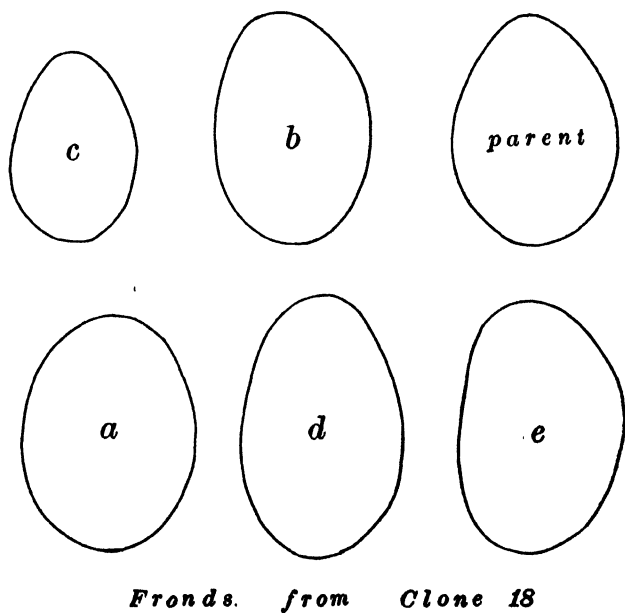
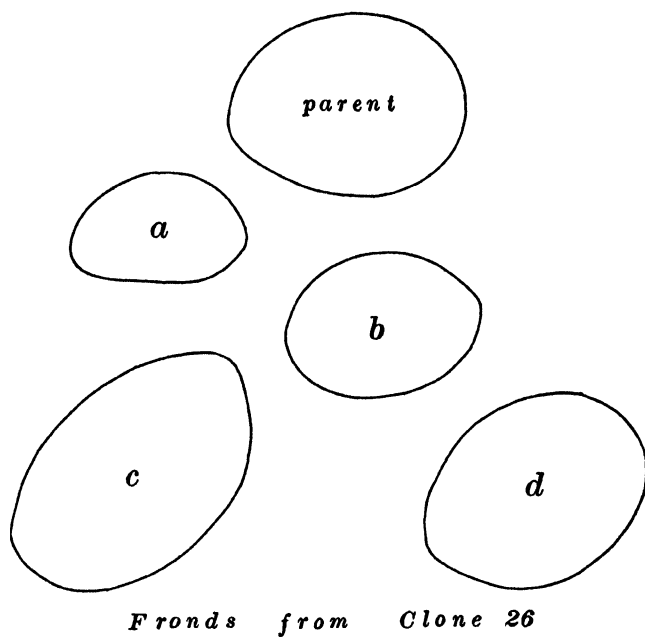


FIGURE 3.—Fronds from clones 18 and 26. $\times 16$ diameters.

of new individuals to be produced from an original frond, or by determining the number of individuals produced within a given length of time. The latter method is simpler and was used in this study.

In this experiment it is necessary that the starting fronds be of the same age. In this and in all other cases where there was necessity of using individuals of the same age, a number of fronds from which no bud had yet appeared were selected from the stock. These were then observed and all fronds appearing for the first time on the same day were taken to be of similar age. By increasing the initial number of starting fronds almost any reasonable number of similar-aged buds could be obtained.

To determine the variation in the speed of propagation, each of a number of buds of the same age from which the first buds appeared at the same time was placed in a culture tumbler and there allowed to propagate. After a certain number of days, the total number of fronds in each tumbler was counted.

Table I contains the results obtained from three determinations and gives a rough idea of the degree of variation in the speed of reproduction.

TABLE I
Variation in speed of reproduction.

Class values	Frequency		
	Dec. 30-Jan. 9	Feb. 20-Mar. 2	Feb. 25-Mar. 7
3	0	0	0
4	5	2	3
5	8	8	3
6	14	8	15
7	22	26	30
8	7	10	4
9	3	5	2
10	1	1	3
Mean	6.517 \pm .115	6.883 \pm .110	6.783 \pm .107
σ	1.323 \pm .081	1.266 \pm .078	1.266 \pm .075
C. V.	20.30 \pm 1.30	18.39 \pm 1.16	18.07 \pm 1.14

The variations shown in the preceding table do not appear to be multimodal and do not indicate that they represent different speed strains.

Variation in the habit of root growth

It is commonly observed that there is a tendency for plants of *L. minor* to produce curly or twisted roots. The manner of this curling or twist-

ing is by no means uniform. While in general the curling is only immediately below the tip, other plants have longer portions of their roots in a twisted condition. In a few cases, the twisting may even come to the middle of the root.

The value of this habit of the plant as a character for the study of variation depends upon whether it is hereditary or is merely the effect of environment.

Unfortunately, variation in this character cannot be measured with any degree of accuracy and does not lend itself readily to genetical study. What is worse, it makes the study of the variation in size, such as in length of the roots almost impossible. An attempt was made to grow a number of the duckweeds on 2 percent agar-containing nutrient solution, hoping to get straight roots which would lend themselves to measurements, but this attempt failed, the roots refusing to grow or sink into the agar media.

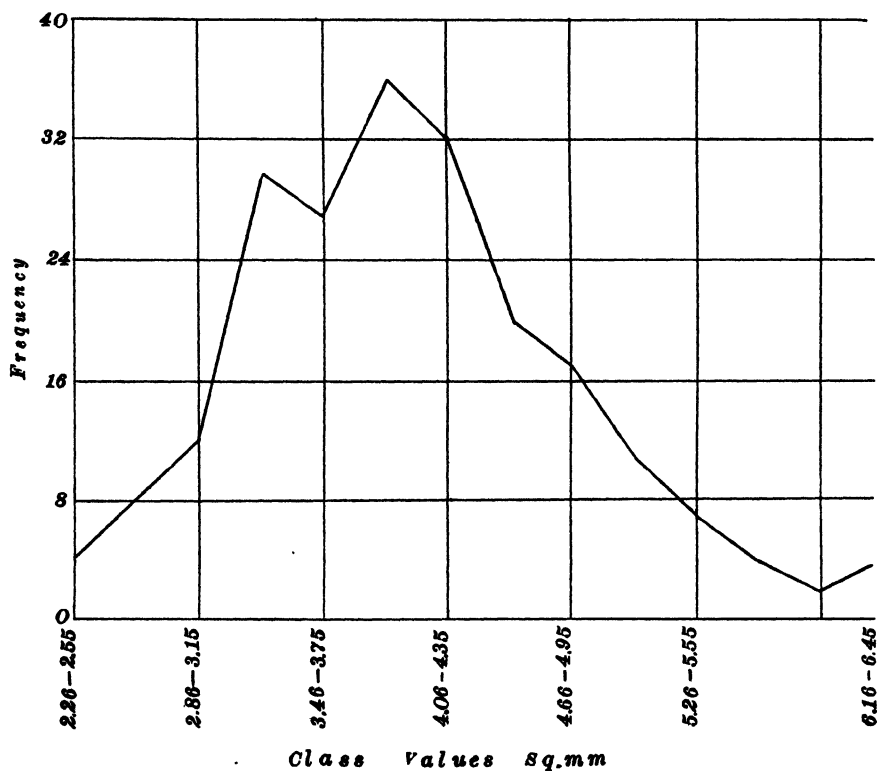


FIGURE 4.—Curve showing variation in size in a population of *L. minor*.

Variation in size of fronds

The size of a frond was determined by measuring its camera-magnified area with the aid of a small planimeter and then computing the true area by dividing the magnified area by 256, the number of times the object was magnified. Two hundred mature-population fronds which were of the same age and which matured at the same time were so measured. Table 2 gives the results of the measurement and figure 4 shows the frequency curve. The curve shows a tendency to three modes, one of these occurring at 3.16-3.45 mm², another at 3.76-4.05 mm², and a third at 6.16-6.45 mm². It might be concluded from this that in a population of *L. minor* there is a probability of the existence of diverse size strains. Such diverse strains need not be found in all localities since the extreme rareness with which this plant has a chance to cross-breed and the rapidity with which it reproduces by budding, both tend, with the help of natural selection, to reduce the inhabitants of a locality to that of a clonal line. None of the clonal lines studied showed a bimodal condition.

Following the determination of the frequency distribution shown in table 2 it would have been only logical to ascertain whether the size modes persist, that is, whether or not the size races found are permanent. An attempt was made to do this. It was planned to isolate several lines representing widely different sizes and then to determine at different intervals of time the average of each line. This attempt, however, was unsuccessful. It was found that *L. minor* cannot be grown successfully in tap water for several months in spite of frequent change of this medium. After a month or so, the fronds usually begin to decrease in size and by the time when enough individuals are needed to give a fair sample, the lines usually have run out. As will be learned later in this paper, continuous culture was maintained by the use of a mineral nutrient solution. It was deemed unwise, however, to use this culture in such an experiment as the determination of the persistence of size differences, since, as will soon be seen, mineral solution had a decided effect in increasing the size of the fronds and no form of culture check could be devised with which this effect could be controlled.

TABLE 2
Distribution of variation in the size of 200 fronds from a wild population of Lemna minor.

	Class values in square millimeters														Mean	σ	C. V.
	2.26-2.55	2.56-2.85	2.86-3.15	3.16-3.45	3.46-3.75	3.76-4.05	4.06-4.35	4.36-4.65	4.66-4.95	4.96-5.25	5.26-5.55	5.56-5.85	5.86-6.15	6.16-6.45			
Frequency	3	7	11	29	26	35	31	19	16	10	6	3	1	3	4.019±.037	.767±.026	16.60±.570

VARIATION AND SELECTION IN CLONAL LINES

Variation and selection in shape of frond

It has been seen already, in the discussion of the permanence of shape strains, that different clones with distinctly different-shaped fronds tend to reproduce their respective characteristic shapes. A certain amount of variation in shape within the clones was also pointed out. Further studies along this line were carried out. The plants, as previously, were grown in tumblers, but in mineral nutrient solution instead of tap water. The use of this solution made the continuous change of culture media unnecessary. The nutrient solution was prepared according to the following modified formula of PFEFFER:

Constituents	Grams per liter
$\text{Ca}(\text{NO}_3)_2$	0.4
NaCl	0.1
MgSO_4	0.1
KH_2PO_4	0.1
$\text{Fe}_3(\text{PO}_4)_2$	0.1
KNO_3	0.1

To study the variation in shape, one hundred mature fronds grown

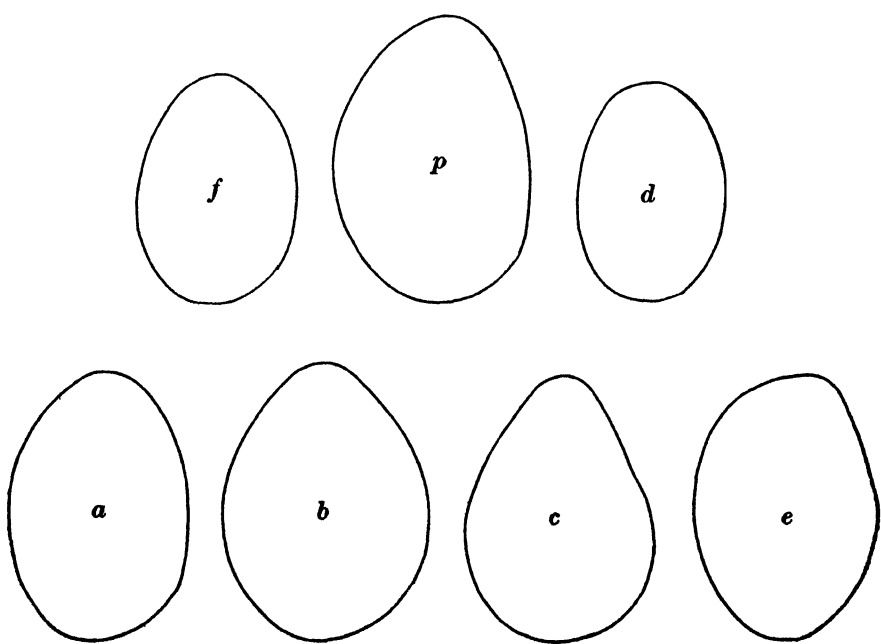


FIGURE 5.—Variation in frond shape in clone 35. $\times 16$ diameters.

from buds of the same age were drawn for each clone. The resulting drawings were classified according to shapes. Figure 5 will give some idea of the dominant shape and the shapes of the varying individuals in clone 35. This dominant shape is represented by the letter p, while the varying shapes are represented by letters a, b, etc.

The frequency of the different shape types in four clones studied is given in table 3.

TABLE 3
Frequency of shape variants.

Clone	Shape types	Total number of individuals
35	p a b c d e f	
	51 3 22 17 1 2 4	100
38	Shape types p g h i j k l	
	47 21 13 11 5 2 1	100
76	Shape types p m n o q r	
	48 7 10 15 18 2	100
81	Shape types p s t u v w	
	46 14 13 13 1 13	100

From table 3 it may be seen that in clonal lines there exist different shapes of fronds, with some one type predominating.

Before taking up the subject of inheritance in shape in clonal lines, it is well to discuss the results of the study of several of the factors affecting variation.

Effect of culture media

Before this part of the experiment was undertaken, it had been observed that fronds growing in tap water had a decidedly different appearance from those growing in nutrient solution. This was partly due to a difference in size; those growing in nutrient medium were very much larger than those in tap water. Suspecting that there may be also a difference in general shape in the two cultures, it was decided to carry out experiments along this line. Clones 38, 39, 41 and 79 were used. Parallel cultures were set up for each clone. Initial buds of those grown

in tap water came from stock growing in tumblers containing water and garden soil, while buds of those grown in nutrient solution came from stocks already growing in nutrient medium. The four series were not grown at the same time as were the paired cultures from each clone. From each culture one hundred mature fronds were harvested, drawn, and classified according to shape. Table 4 gives the frequency of the different types observed.

TABLE 4
Frequency of shape types of plants grown in tap water and in nutrient solution.

Clone 38			Clone 39			Clone 41			Clone 79		
Types	Tap water	Nutrient solution	Types	Tap water	Nutrient solution	Types	Tap water	Nutrient solution	Types	Tap water	Nutrient solution
a	18	28	i	24	36	o	0	8	u	27	41
b	20	3	j	16	18	p	49	12	v	15	17
c	7	12	k	0	17	q	28	54	w	10	10
d	2	1	l	12	4	r	16	11	x	0	1
e	33	7	m	8	13	s	7	9	y	48	20
f	0	1	n	40	12	t	0	6	z	0	11
g	0	3									
h	20	45									
Total number	100	100		100	100		100	100		100	100

Table 4 shows two important points: (1) in every case there was found greater variation in nutrient-grown plants than in those grown in tap water, and (2) the predominant shape in each clone is different for the two culture media. In clone 38, for example, shape e was predominant among the tap-water-grown plants while among those grown in nutrient solution shape h was the predominating type.

Inheritance of shape within a clone

It has already been pointed out (see table 3) that a study of one hundred mature fronds of clone 81 revealed six shape types, s to w, with type p predominating. To determine to what extent these different shape types are hereditary, a family was bred from each type in nutrient solution and one hundred mature fronds from each were drawn and studied as to variation in shape. Table 5 contains the results of this study.

We see from table 5 that the parental type seems to have had no effect on the type distribution (excepting the type representing the clone. An interesting fact brought out by the above data is that while the diverse shapes which do not represent that of the clone were not hereditary, they

TABLE 5
Frequency of types in different families of clone 81.

Parent types	Types of progeny and their distribution										
	s	t	p	u	v	w	g	h	i	j	k
s	1	7	43	13	1	17	0	6	2	6	4
t	0	6	36	17	1	9	7	12	1	5	6
p	3	8	51	9	1	9	5	5	1	6	2
u	1	6	47	3	0	30	0	4	1	8	0
v	1	6	47	9	2	19	3	8	1	3	1
w	2	8	41	7	1	25	2	10	1	3	0

appeared in approximately the same relative proportion to one another irrespective of their parental shapes.

In order that this point may be seen more clearly, the data in table 5 were made into curves shown in figure 6.

Another attempt to change the dominant shape type of clone 81 was made by continuous selection of shapes u and w. The experiment was carried through three periods, each period comprising many generations. There were three cultures during each period, one for u selection, one for w and another for p. The latter served as control. One hundred mature fronds were examined from each harvest. Table 6 contains the results.

TABLE 6
Results of continuous selection for types u and w in clone 81.

Parent shapes	Shapes of progeny and distribution										
	s	t	p	u	v	w	g	h	i	j	k
First period											
u	1	5	49	10	2	25	1	2	2	3	0
p	3	5	48	11	2	21	1	2	2	5	0
w	7	3	46	4	2	20	2	7	2	7	0
Second period											
u	6	12	38	5	0	27	0	10	2	0	0
p	3	13	35	5	1	31	3	8	1	0	0
w	10	17	26	4	2	22	6	7	6	0	0
Third period											
u	17	0	50	12	5	8	3	2	3	0	0
p	13	7	37	18	3	22	0	0	0	0	0
w	7	5	29	16	7	17	8	7	4	0	0

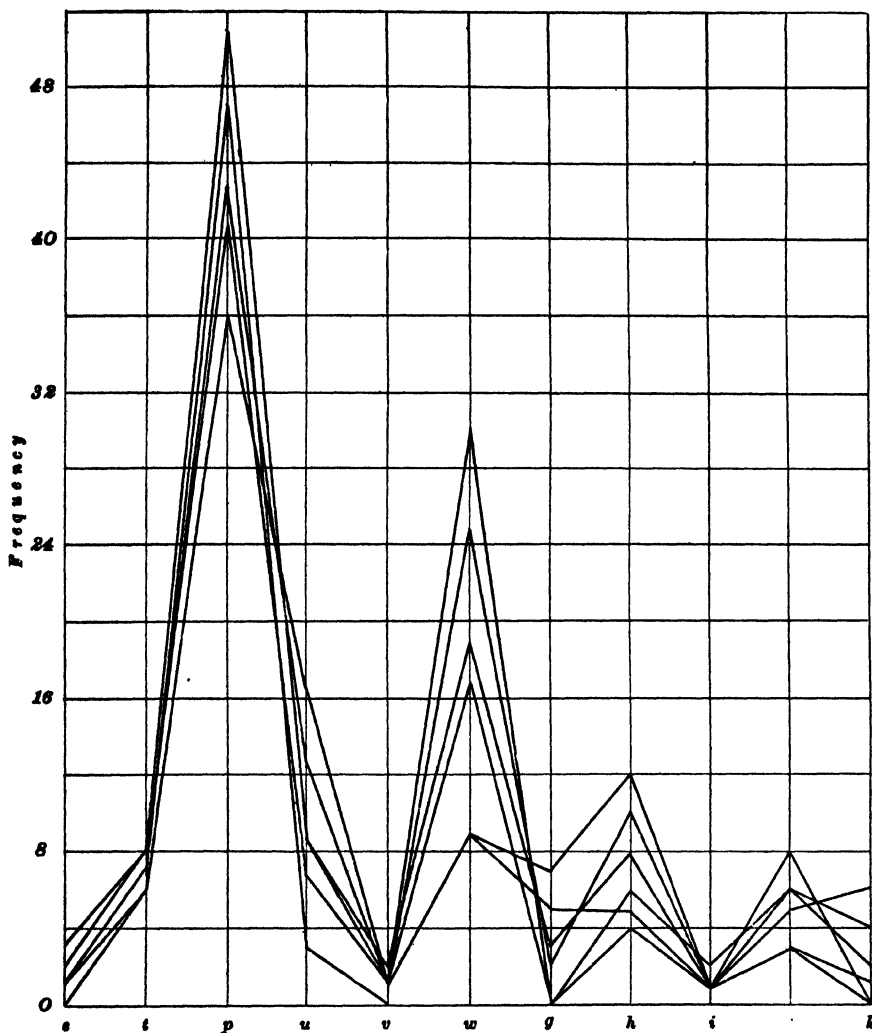


FIGURE 6.—Frequency curves of different shape types in different families of clone 81.

It would appear from the results shown in tables 5 and 6 that the different non-dominant shape types in clone 81 are merely somatic variations, probably physiological, and are not inheritable, and that selection for these different shapes has made no progress. As early as 1894, GUPPY (1894) reported that long exposure to different habits of life, as growing in mud, had not produced any permanent change in the external appearance of duckweeds.

Unusual non-heritable variations in frond shape

During the entire period of investigation, a watch was continually kept for mutations. Three fronds of unusual shapes appeared in nutrient cultures, two in clone 41 and one in clone 42. When they were found, they were still attached to their parent fronds. Each of these unusual-shaped fronds together with each parent was placed in a separate culture tumbler and allowed to propagate to determine if they were mutations. When matured individuals in each tumbler numbered fifty or more, the cultures were discontinued and the mature fronds examined. It was found that none of these aberrant shapes was hereditary.

Selection in opposite directions was made in each of the four clones mentioned above. Each selection was carried through five periods. Plus selection was made by continuous selection of individuals falling in classes 9 and 10, and minus selection, of those in classes 5 and 4. A check culture of unselected individuals was also grown. The three cultures in each clone—plus, minus and check,—were always grown at the same time. The plants were grown in the nutrient medium and good care was taken to render cultural and other controllable conditions as much alike as possible for each series. Tables 8-11, inclusive, show the results of this selection, and table 12 contains the differences between the means of the check cultures and those of the plus and minus selections. If the selection be effective, there should be an increasing difference between the means of the check and selection series from the first to the last period of the experiment.

Clonal variation and selection in speed of propagation

In this study, clones 38, 39, 78 and 81 were used. The unit of time taken was 11 days. Sixty individuals were studied in each culture. Initial studies of variation in speed of propagation of these different lines gave results which are shown in table 7. A "class value" in this case represents the total number of individuals obtained by allowing an original bud and its offspring to propagate during 11 days.

TABLE 7
Clonal variation in speed of propagation.

Clone	Period	Class values										Mean	σ	C.V.
		3	4	5	6	7	8	9	10	11				
38	May 18-June 18	0	3	6	8	23	11	8	1	0	7.017 \pm .118	1.360 \pm .084	19.38 \pm 1.24	
39	May 18-June 18	1	2	2	6	24	11	8	5	1	7.433 \pm .133	1.532 \pm .094	20.61 \pm 1.32	
78	June 11-June 21	0	3	7	11	25	10	3	1	0	6.750 \pm .109	1.247 \pm .077	18.47 \pm 1.17	
81	June 19-June 29	0	2	3	12	27	10	4	2	0	7.000 \pm .104	1.197 \pm .074	17.10 \pm 1.08	

From table 7 it is to be seen that clones 38 and 81 seem to have the same speed of budding. The other two clones, however, appear to possess distinctly different means, whose difference is $.683 \pm .172$, so that it may be considered as highly probable that in a population of *Lemna minor*, there exist also different strains in regard to speed of asexual propagation.

TABLE 8
Selection in speed of propagation in clone 38.

Selection	Frequency of class values										Mean	σ	C.V.
	3	4	5	6	7	8	9	10	11				
First period													
Plus (+)			2	7	15	26	8	2			6.617±.093	1.066±.066	16.11±1.02
Check			1	7	14	27	9	2			6.700±.082	1.021±.063	15.24±0.96
Minus (—)			1	10	12	25	10	2			6.650±.095	1.093±.067	16.44±1.04
Second period													
Plus (+)				8	15	28	6	3			6.683±.086	0.991±.061	18.83±0.93
Check				7	15	27	8	3			6.750±.087	0.994±.061	14.72±0.92
Minus (—)			3	8	17	23	6	3			6.500±.100	1.147±.071	17.65±1.12
Third period													
Plus (+)			3	8	13	26	8	2			6.567±.098	1.131±.070	17.22±1.09
Check			1	11	15	26	5	2			6.483±.091	1.041±.064	16.06±1.01
Minus (—)			3	6	14	25	10	2			6.650±.098	1.123±.069	16.89±1.07
Fourth period													
Plus (+)			3	4	10	25	12	6			6.950±.106	1.217±.075	17.52±1.11
Check			3	8	10	25	11	2	1		6.717±.108	1.240±.076	18.46±1.17
Minus (—)			2	4	11	24	11	5	3		7.083±.114	1.308±.081	18.47±1.17
Fifth period													
Plus (+)			2	8	12	26	9	2	1		6.700±.102	1.173±.072	17.51±1.11
Check			1	10	14	25	7	3			6.600±.096	1.098±.068	16.64±1.05
Minus (—)			3	9	13	24	9	2			6.550±.101	1.161±.071	17.72±1.12

TABLE 9

Results of continuous selection in speed of propagation in clone 39.

Selection	Frequency of class values												Mean	σ	C. V.
	3	4	5	6	7	8	9	10	11	12					
First period															
Plus (+)			3	3	7	30	8	6	3				7.117±.115	1.318±.081	18.52±1.18
Check			2	4	4	30	8	8	3	1			7.317±.121	1.384±.085	18.91±1.20
Minus (—)			2	6	6	29	10	4	2	1			7.067±.117	1.340±.083	18.96±1.21
Second period															
Plus (+)		1	4	6	28	11	9	1					6.250±.103	1.178±.073	18.85±1.20
Check		2	2	5	29	9	7	5	1				6.450±.122	1.396±.086	21.64±1.39
Minus (—)		3	3	4	27	12	10	1					6.267±.112	1.289±.079	20.57±1.32
Third period															
Plus (+)			3	2	8	26	10	6	4	1			7.283±.125	1.439±.089	19.76±1.26
Check		1	2	3	9	25	9	6	5				7.183±.129	1.478±.091	20.58±1.32
Minus (—)			3	4	7	26	8	7	4	1			7.233±.131	1.499±.092	20.72±1.33
Fourth period															
Plus (+)			1	2	8	28	9	6	5	1			7.417±.117	1.345±.083	18.13±1.15
Check		2	0	3	6	29	10	7	3				7.217±.119	1.367±.084	18.94±1.21
Minus (—)		1	4	4	5	31	7	5	2	1			6.967±.130	1.494±.092	21.44±1.38
Fifth period															
Plus (+)				1	8	21	14	6	7	3			7.817±.124	1.420±.087	18.16±1.15
Check		1	0	3	3	25	10	4	7	5	2		7.933±.158	1.815±.112	22.87±1.48
Minus (—)			2	3	4	25	10	8	5	3			7.617±.136	1.561±.096	20.49±1.31

TABLE 10

Results of continuous selection in speed of propagation in clone 78.

Selection	Frequency of class values										Mean	σ	C.V.
	3	4	5	6	7	8	9	10	11				
First period													
Plus (+)			1	7	21	22	7	2			6.550±.088	1.007±.062	15.37±0.97
Check			1	10	21	25	3	0			6.317±.075	0.866±.053	13.71±0.86
Minus (—)			2	10	16	27	3	2			6.417±.090	1.038±.064	16.17±1.02
Second period													
Plus (+)			1	7	11	25	11	4	1		6.900±.103	1.179±.073	17.09±1.08
Check	1		3	5	6	29	9	5	2		6.933±.121	1.389±.086	20.03±1.28
Minus (—)			2	6	10	30	7	4	1		6.833±.102	1.171±.072	17.13±1.08
Third period													
Plus (+)				5	17	31	5	2			6.700±.075	0.862±.053	12.86±0.80
Check	1	2		6	14	27	8	2			6.600±.100	1.143±.070	17.32±1.10
Minus (—)			5	7	13	24	8	3			6.533±.108	1.245±.077	19.06±1.21
Fourth period													
Plus (+)			1	5	10	29	11	3	1		6.950±.095	1.087±.067	15.64±0.99
Check			1	3	7	31	12	4	2		7.167±.102	1.171±.072	16.33±1.03
Minus (—)			2	4	10	27	12	4	1		6.983±.101	1.162±.072	16.64±1.05
Fifth period													
Plus (+)			2	5	9	26	13	3	2		7.000±.107	1.225±.075	17.50±1.11
Check	1		1	5	8	28	12	4	1		6.967±.107	1.224±.075	17.57±1.11
Minus (—)			1	8	8	30	10	2	1		6.833±.097	1.113±.069	16.29±1.03

TABLE II

Results of continuous selection in speed of propagation in clone 81.

Selection	Frequency of class values										Mean	σ	C.V.
	3	4	5	6	7	8	9	10	11				
First period													
Plus (+)	5	7	10	13	22	2	1				5.833±.123	1.416±.087	24.27±1.58
Check	6	8	10	13	20	2	1				5.717±.127	1.462±.090	25.57±1.67
Minus (—)	8	10	12	23	5	2					5.217±.113	1.292±.080	24.76±1.61
Second period													
Plus (+)	2	3	5	12	30	4	3	1			6.567±.115	1.321±.081	20.12±1.29
Check		1	5	6	33	8	3	3	1		7.133±.111	1.271±.078	17.82±1.13
Minus (—)	1	3	4	7	34	6	3	2			6.833±.113	1.293±.080	18.92±1.20
Third period													
Plus (+)			2	1	10	25	13	5	4		7.283±.110	1.266±.078	17.38±1.10
Check			1	2	11	28	10	6	1	1	7.183±.104	1.190±.073	16.57±1.05
Minus (—)			1	4	7	31	9	5	2	1	7.183±.108	1.245±.077	17.33±1.10
Fourth period													
Plus (+)				1	4	17	26	6	5	1	6.850±.098	1.123±.069	16.39±1.03
Check				1	5	20	24	5	4	1	6.717±.097	1.112±.068	16.55±1.05
Minus (—)				1	2	12	28	10	5	2	7.117±.098	1.127±.069	15.83±1.00
Fifth period													
Plus (+)				1	4	6	32	9	5	2	7.200±.108	1.236±.076	17.16±1.09
Check				1	3	10	29	8	6	2	7.183±.110	1.258±.077	17.51±1.11
Minus (—)				1	5	5	30	8	7	3	7.283±.117	1.343±.083	18.44±1.17

TABLE 12
Differences between the means of the check and those of the selections.

Period	Difference between means of check and plus selections	Difference between means of check and minus selections
Clone 38		
First	-0.083 ± .124	0.050 ± .125
Second	-0.067 ± .122	0.250 ± .133
Third	0.084 ± .134	-0.167 ± .134
Fourth	0.233 ± .151	-0.366 ± .157
Fifth	0.100 ± .140	0.050 ± .139
Clone 39		
First	-0.200 ± .167	0.250 ± .168
Second	-0.200 ± .160	0.183 ± .166
Third	0.100 ± .179	-0.050 ± .184
Fourth	0.200 ± .167	0.250 ± .176
Fifth	-0.116 ± .201	0.316 ± .209
Clone 78		
First	0.233 ± .115	-0.100 ± .117
Second	-0.033 ± .159	0.100 ± .158
Third	0.100 ± .125	0.067 ± .147
Fourth	-0.217 ± .139	0.184 ± .144
Fifth	0.033 ± .151	0.134 ± .144
Clone 81		
First	0.116 ± .177	0.500 ± .170
Second	-0.566 ± .160	0.300 ± .158
Third	0.100 ± .151	0.000 ± .150
Fourth	0.133 ± .138	-0.400 ± .138
Fifth	0.017 ± .154	-0.100 ± .161

From the data in table 12 it may be concluded that there was no progress obtained in either the plus or minus selection for speed of budding.

Clonal variation in size of frond

As a preliminary selection study the variation in size of fronds in four clones was studied. Selection was later performed in these same four lines. The plants were grown in nutrient solution contained in

TABLE 13
Variation in size in square millimeters in clonal lines of Lemna minor.

Clone	Class values and frequencies															Mean	σ	C. V.						
	1.76-2.25	2.26-2.75	2.76-3.25	3.26-3.75	3.76-4.25	4.26-4.75	4.76-5.25	5.26-5.75	5.76-6.25	6.26-6.75	6.76-7.25	7.26-7.75	7.76-8.25	8.26-8.75	8.76-9.25				9.26-9.75	9.76-10.25	10.26-10.75	10.76-11.25	11.26-11.75	11.76-12.25
38				1	3	8	6	17	11	11	10	10	7	9	5	1	0	1				6.555 \pm .098	1.460 \pm .070	20.75 \pm 1.03
76				1	4	6	9	8	20	10	6	8	6	6	6	3	1	1				6.735 \pm .112	1.605 \pm .079	24.72 \pm 1.25
79			1	2	2	9	11	18	11	11	15	10	4	1	1	1	1	1	0	0		6.220 \pm .099	1.472 \pm .070	23.66 \pm 1.20
81						4	6	12	5	13	11	17	12	12	2	4	4	2				7.075 \pm .090	1.334 \pm .064	18.85 \pm 0.93

tumblers. One hundred mature fronds were measured from each clone. The results of this study are shown in table 13. From table 13 it is seen that clones 38 and 76 have about the same range of variation. They also approach each other in mean size, which is $6.555 \pm .098 \text{ mm}^2$ in clone 38 and $6.735 \pm .112 \text{ mm}^2$ in clone 76. The standard deviations are $1.460 \pm .070$ and $1.665 \pm .079 \text{ mm}^2$ respectively. Clone 79 has the widest range of variation and the least mean size, $6.220 \pm .099 \text{ mm}^2$. Its standard deviation is $1.472 \pm .070 \text{ mm}^2$. Clone 81 has the largest mean size, $7.075 \pm 0.090 \text{ mm}^2$, and the least standard deviation, $1.334 \pm .064 \text{ mm}^2$. Clones 79 and 81 show a significant difference. The difference in the mean is $.855 \pm .134$.

TABLE 14
Variation in size of fronds grown in nutrient solution and in tap water.

Clone	Period	Culture media	Frequency of class values in square millimeters (mm ²)																		
			1.76-2.25	2.26-2.75	2.76-3.25	3.26-3.75	3.76-4.25	4.26-4.75	4.76-5.25	5.26-5.75	5.76-6.25	6.26-6.75	6.76-7.25	7.26-7.75	7.76-8.25	8.26-8.75	8.76-9.25	9.26-9.75	9.76-10.25	10.26-10.75	10.76-11.25
38	First	Nutrient				1	1	4	2	6	14	12	12	15	10	7	6	4	3	2	1
		Tap water	4	13	10	10	24	14	18	6	1										
	Second	Nutrient					2	7	6	11	24	20	11	11	7	1					
		Tap water		1	5	12	22	25	24	8	3										
	Third	Nutrient					6	1	11	14	21	14	8	10	6	4	4	1			
		Tap water	2	7	14	16	20	23	11	5	1	1									
41	First	Nutrient							2	3	12	14	20	11	9	12	8	4	3	0	2
		Tap water		5	14	8	17	18	14	8	11	2	1								
	Second	Nutrient				2	1	4	14	16	19	13	11	9	4	7					
		Tap water	3	7	8	20	19	23	13	4	2	1									
	Third	Nutrient			1	2	2	1	6	3	14	22	16	9	11	5	5	1	1		
		Tap water	1	4	9	14	18	15	13	5	10	3	6	1	0	1					
79	First	Nutrient					1	2	5	7	14	6	13	11	6	13	9	9	1	2	1
		Tap water	1	3	3	8	19	20	12	17	13	3	1								
	Second	Nutrient					3	7	12	10	16	11	11	6	6	5	5	3	3	2	
		Tap water	13	7	11	15	8	13	19	6	6	0	2								
	Third	Nutrient			1	1	3	3	7	7	12	13	11	12	8	11	5	3	0	2	1
		Tap water	2	9	13	20	27	19	7	1	1	1									
81	First	Nutrient			1	0	2	5	5	8	10	14	20	15	8	4	2	4	1	0	1
		Tap water	6	8	19	17	17	23	4	3	2	1									
	Second	Nutrient					2	4	4	6	12	16	19	11	8	3	6	6	2	1	
		Tap water	1	5	15	13	20	19	13	9	4	1									
	Third	Nutrient				2	4	2	8	14	8	11	6	11	10	8	8	2	4	1	1
		Tap water	4	9	15	17	14	24	8	2	6	1									

Effect of culture media on clonal size variation

In the study of inheritance of acquired size in *Lemna minor*, which is reported later on in this paper, parallel cultures were grown from each clone in nutrient solution and in tap water. The materials obtained from this experiment may also be examined for the effect of different culture media on variation in size. From each of the four clones used, 38, 41, 79 and 81, three series were grown in different periods of time. One hundred mature fronds were measured from each culture. The results of these measurements are given in tables 14 and 15, the former gives the frequency distributions of the different classes found, and the latter, the different constants calculated.

TABLE 15
Constants from table 14.

Clone	Period	Culture medium	Mean	C.V.	σ	$\sigma_{\text{nut}} - \sigma_{\text{tap}}$
38	First	Nutrient	7.210 \pm .102	20.87 \pm 1.04	1.505 \pm .072	0.524 \pm .086
		Tap water	4.070 \pm .066	24.10 \pm 1.21	0.981 \pm .047	
	Second	Nutrient	6.285 \pm .067	15.88 \pm 0.77	0.998 \pm .047	0.271 \pm .058
		Tap water	4.425 \pm .049	16.43 \pm 0.80	0.727 \pm .035	
	Third	Nutrient	6.365 \pm .085	19.81 \pm 0.98	1.261 \pm .060	0.367 \pm .074
		Tap water	3.985 \pm .060	22.43 \pm 1.12	0.894 \pm .043	
41	First	Nutrient	7.485 \pm .086	17.01 \pm 0.83	1.273 \pm .061	0.198 \pm .079
		Tap water	4.430 \pm .073	24.27 \pm 0.93	1.075 \pm .051	
	Second	Nutrient	6.220 \pm .078	18.55 \pm 0.91	1.154 \pm .054	0.245 \pm .069
		Tap water	4.030 \pm .061	22.55 \pm 1.13	0.909 \pm 0.43	
	Third	Nutrient	6.985 \pm .088	18.74 \pm 0.92	1.309 \pm .062	0.022 \pm .087
		Tap water	4.575 \pm .087	28.13 \pm 1.44	1.287 \pm .061	
79	First	Nutrient	7.145 \pm .103	21.53 \pm 1.07	1.538 \pm .073	0.533 \pm .087
		Tap water	4.710 \pm .068	21.34 \pm 1.06	1.005 \pm .048	
	Second	Nutrient	6.635 \pm .108	24.08 \pm 1.21	1.598 \pm .076	0.332 \pm .097
		Tap water	3.980 \pm .085	31.81 \pm 1.66	1.266 \pm .060	
	Third	Nutrient	6.925 \pm .106	22.71 \pm 1.14	1.573 \pm .075	0.753 \pm .084
		Tap water	3.825 \pm .055	21.44 \pm 1.07	0.820 \pm .039	
81	First	Nutrient	6.815 \pm .093	20.32 \pm 1.01	1.385 \pm .066	0.438 \pm .080
		Tap water	3.755 \pm .064	25.22 \pm 1.28	0.947 \pm .045	
	Second	Nutrient	7.020 \pm .095	20.03 \pm 0.99	1.406 \pm .067	0.450 \pm .081
		Tap water	4.160 \pm .064	22.98 \pm 1.15	0.956 \pm .046	
	Third	Nutrient	6.940 \pm .114	24.38 \pm 1.23	1.692 \pm .081	0.674 \pm .095
		Tap water	3.930 \pm .069	25.90 \pm 1.31	1.018 \pm .049	

The outstanding result shown by table 15 is that, using standard deviation as the expression of variation, the plants grown in nutrient solution were always more variable in size than those grown in tap water. The differences between the standard deviations of parallel cultures are significant and are, with two exceptions, all well beyond the limits of probable error.

Inheritance of acquired size

The fact has already been pointed out that plants growing under natural conditions have demonstrated their capacity to react readily with favorable medium for growth, not only by a change in shape of the fronds but also by a considerable increase in size, amounting in some cases to more than 100 percent. Likewise it was observed that fronds previously grown in nutrient solution produced offspring which are very much smaller than themselves.

Inheritance of decreased size

An experiment to determine the inheritance of decreased size was made with clones 38 and 41 as follows: From a stock culture of each clone, the same number of buds of the same age were transferred to both tap water and nutrient media and there allowed to propagate until a sufficient harvest of mature fronds could be obtained at any one time. This constitutes the first period of the experiment. In the second period, cultures in both tap water and nutrient solutions were grown from buds from the tap water culture of the first period. At the same time a check culture in nutrient medium was grown. In the third period, tap water and nutrient cultures were similarly grown from the tap water stock of the preceding period and again a check culture was set. There are several months of interval between each two periods to give the plants time to be "acclimatized" in each new medium for growth. From each of

TABLE 16

Mean size in square millimeters of fronds from nutrient solution, grown in tap water, and of their offspring when grown again in nutrient solution.

Clone	Parent mean in nutrient solution	Offspring mean in tap water	Mean when back in nu- trient solution	Check in nutrient solution
38	6.555 \pm .098	4.070 \pm .066	6.315 \pm .066	6.775 \pm .091
		4.425 \pm .049	6.365 \pm .085	6.450 \pm .092
41	7.120 \pm .073	4.430 \pm .073	6.220 \pm .078	6.615 \pm .062
		4.030 \pm .061	6.805 \pm .090	6.985 \pm .088

these cultures one hundred fronds were measured. Table 16 contains a summary of the results of this experiment.

From table 16 it may be concluded that decreased size acquired by nutrient fronds in their sojourn in tap water is not inherited.

Inheritance of increased size

The plan of this experiment is inversely similar to that of the inheritance of decreased size.

This experiment was carried through only two periods. As usual, one hundred mature fronds were studied from each culture. The results of the measurements are shown in table 17.

TABLE 17

Mean sizes in square millimeters of tap-water fronds grown in nutrient solution and of their offspring when grown in tap water.

Clone	Parent mean in tap water	Offspring mean in nu- trient solution	Mean when back in tap water	Check in tap water
79	4.750 \pm .068	7.405 \pm .104	3.980 \pm .085	3.825 \pm .055
81	3.990 \pm .066	6.815 \pm .093	4.160 \pm .064	3.930 \pm .069

From table 17 it is seen that while starved plants grown in nutrient media increase in size by nearly 100 percent, when grown again in tap water reversion to the starved mean may be complete, showing no inheritance of the acquired increased size.

Clonal selection for size of frond

In table 13 the variations in size of clones 38, 76, 79 and 81 have already been shown. Selections for both large and small size were carried out with these four clones as an attempt to shift the means of the lines. Each selection was carried through five periods. Plus selection, or selection for large size, was made by continuously selecting individuals above the mean, and minus selection, or selection for small size, was performed by continuous selection of individuals below the mean. A check culture containing unselected individuals was also grown at the same time with the plus and minus series. The plants were grown in nutrient solution and extreme care was taken to render all controllable conditions as much alike as possible for each set of three cultures. Tables 18 to 21 contain the results of this experiment. The differences between the means of the check cultures and those of the plus and minus selections are placed in table 22 so that the effect of selection may be studied more conveniently.

TABLE 18
Results of continuous selection for large and small size in Clone 38.

Selection	Frequency of class values in square millimeters																								Mean	σ	C.V.				
1.76 to 2.25	2.26 to 2.75	2.76 to 3.25	3.26 to 3.75	3.76 to 4.25	4.26 to 4.75	4.76 to 5.25	5.26 to 5.75	5.76 to 6.25	6.26 to 6.75	6.76 to 7.25	7.26 to 7.75	7.76 to 8.25	8.26 to 8.75	8.76 to 9.25	9.26 to 9.75	9.76 to 10.25	10.26 to 10.75	10.76 to 11.25	11.26 to 11.75	11.76 to 12.25											
First period																															
Plus (+)					6	4	14	18	16	18	13	6	2	2	0	1					6.530 ± .072	1.073 ± .051	16.43 ± 0.80								
Check			2	4	4	3	10	22	22	11	6	5	4	3	2	1					6.500 ± .093	1.383 ± .066	21.28 ± 1.06								
Minus (—)				3	5	5	8	13	21	17	15	8	3	2		0	0	1			6.575 ± .075	1.107 ± .053	16.83 ± 0.82								
Second period																															
Plus (+)					3	5	9	11	14	13	9	10	9	7	3	1	1				6.485 ± .103	1.525 ± .073	23.51 ± 1.18								
Check		2	1	1	5	7	9	11	13	19	13	9	5	4	0	1	2				6.775 ± .091	1.366 ± .065	20.16 ± 1.00								
Minus (—)			1	4	5	10	13	15	9	10	13	7	1	1	1	1	0	1			6.535 ± .098	1.451 ± .069	22.20 ± 1.11								
Third period																															
Plus (+)					2	4	7	15	20	22	13	9	3	4	0	1					6.830 ± .069	1.026 ± .049	15.02 ± 0.73								
Check				1	1	6	13	16	22	19	10	4	3	3	1	1					6.615 ± .072	1.074 ± .051	16.23 ± 0.70								
Minus (—)			2	0	2	5	11	11	24	23	11	8	1	1	1						6.585 ± .069	1.022 ± .049	15.52 ± 0.70								
Fourth period																															
Plus (+)					1	0	3	2	3	14	18	20	15	11	5	4	3	1			7.025 ± .078	1.157 ± .055	16.47 ± 0.81								
Check				2	4	1	7	12	14	23	15	7	7	4	3	1					6.965 ± .082	1.218 ± .058	17.49 ± 0.86								
Minus (—)		1	0	0	2	4	5	15	18	28	18	3	5	0	0	0	0	1			6.755 ± .069	1.019 ± .049	15.08 ± 0.73								
Fifth period																															
Plus (+)					2	4	4	1	5	9	10	18	19	7	7	5	4	3			7.535 ± .081	1.202 ± .057	15.95 ± 0.78								
Check				1	1	4	2	6	14	12	15	10	7	6	4	3	2	1			7.210 ± .102	1.505 ± .072	20.87 ± 1.04								
Minus (—)		2	1	4	3	3	6	9	11	10	12	8	14	7	6	3	0	0	1		7.175 ± .115	1.609 ± .081	23.68 ± 1.19								

TABLE 19
Results of continuous selection for large and small size in clone 76.

Selection	Frequency of class values in square millimeters																								Mean	σ	C.V.
	1.76 to 2.25	2.26 to 2.75	2.76 to 3.25	3.26 to 3.75	3.76 to 4.25	4.26 to 4.75	4.76 to 5.25	5.26 to 5.75	5.76 to 6.25	6.26 to 6.75	6.76 to 7.25	7.26 to 7.75	7.76 to 8.25	8.26 to 8.75	8.76 to 9.25	9.26 to 9.75	9.76 to 10.25	10.26 to 10.75	10.76 to 11.25	11.26 to 11.75	11.76 to 12.25						
First period																											
Plus (+)						2	3	5	5	9	13	11	13	16	9	6	5	2	1					7.740 \pm .095	1.413 \pm .067	18.25 \pm 0.90	
Check							4	5	5	12	13	15	16	9	8	6	5	1	1					7.045 \pm .090	1.331 \pm .063	17.41 \pm 0.85	
Minus (-)				1	1	3	4	7	10	15	11	11	11	7	6	6	4	2	1					7.275 \pm .106	1.568 \pm .075	21.55 \pm 1.07	
Second period																											
Plus (+)								2	3	7	9	8	19	15	10	8	7	3	3	3	3	3		8.485 \pm .100	1.478 \pm .070	17.42 \pm 0.85	
Check							2	3	5	8	9	12	14	15	7	9	4	5	4	1				8.070 \pm .104	1.541 \pm .073	19.09 \pm 0.94	
Minus (-)						1	1	3	8	9	10	11	12	20	10	6	4	2	2	1				7.930 \pm .093	1.385 \pm .066	17.46 \pm 0.86	
Third period																											
Plus (+)							1	4	4	5	12	18	14	11	11	7	5	3	2	2	1			7.625 \pm .096	1.425 \pm .068	18.69 \pm 0.92	
Check							1	2	3	10	17	19	25	7	6	4	3	2	1					7.245 \pm .074	1.101 \pm .053	15.20 \pm 0.74	
Minus (-)							1	1	3	7	8	17	13	17	7	9	7	5	4	1				7.330 \pm .091	1.355 \pm .065	18.48 \pm 0.91	
Fourth period																											
Plus (+)						1	2	3	7	11	16	12	9	9	5	2	1	1						6.715 \pm .093	1.377 \pm .066	20.51 \pm 1.02	
Check							4	2	8	13	18	14	8	9	4	4	6	2						6.745 \pm .102	1.509 \pm .072	22.37 \pm 1.12	
Minus (-)						1	4	6	13	11	14	11	11	9	8	4	1	1						6.465 \pm .100	1.483 \pm .071	22.94 \pm 1.15	
Fifth period																											
Plus (+)						1	1	1	1	7	10	11	13	13	14	8	7	5	3	2	1	1	1	7.520 \pm .105	1.552 \pm .074	20.64 \pm 1.02	
Check							1	1	4	5	5	7	18	19	12	13	9	4	1	1				7.465 \pm .084	1.250 \pm .060	16.70 \pm 0.82	
Minus (-)							1	3	8	10	10	17	16	9	8	6	5	3	2	1	1			7.430 \pm .097	1.431 \pm .068	19.26 \pm 0.97	

TABLE 20
Results of continuous selection for

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TABLE 21
Results of continuous selection for large and small size in clone 8r.

Selection	Frequency of class values in square millimeters																								C.V.
	1.76 to 2.25	2.26 to 2.75	2.76 to 3.25	3.26 to 3.75	3.76 to 4.25	4.26 to 4.75	4.76 to 5.25	5.26 to 5.75	5.76 to 6.25	6.26 to 6.75	6.76 to 7.25	7.26 to 7.75	7.76 to 8.25	8.26 to 8.75	8.76 to 9.25	9.26 to 9.75	9.76 to 10.25	10.26 to 10.75	10.76 to 11.25	11.26 to 11.75	11.76 to 12.25	Mean		σ	
First period																									
Plus (+)					4	1	3	7	10	13	17	11	8	8	9	6	1	1	1			7.220 \pm .100	1.477 \pm .070	20.46 \pm 1.02	
Check			1	0	2	5	5	8	10	14	20	15	8	4	2	4	1	0	1			6.815 \pm .093	1.385 \pm .066	20.32 \pm 1.01	
Minus (-)					2	1	7	8	11	8	25	13	8	8	5	3	0	1				6.985 \pm .086	1.279 \pm .061	18.31 \pm 0.90	
Second period																									
Plus (+)			1	2	4	6	3	9	13	12	18	16	9	3	2	1	1					6.550 \pm .002	1.358 \pm .005	20.73 \pm 1.03	
Check				2	2	5	5	12	16	15	19	9	5	6	4							6.500 \pm .083	1.232 \pm .059	18.95 \pm 0.93	
Minus (-)			1	1	2	8	13	9	16	10	14	14	4	3	3	2						6.330 \pm .091	1.342 \pm .064	21.20 \pm 1.05	
Third period																									
Plus (+)					2	8	6	6	15	11	24	12	10	1	3	0	1	1				6.630 \pm .085	1.256 \pm .060	18.94 \pm 0.93	
Check				1	0	3	7	13	25	15	15	6	4	2	1	2	2	1				6.405 \pm .088	1.298 \pm .062	20.26 \pm 1.00	
Minus (-)					2	3	6	9	14	17	16	19	7	5	2							6.670 \pm .074	1.091 \pm .052	16.36 \pm 0.80	
Fourth period																									
Plus (+)					1	2	5	6	11	15	13	11	11	5	4	3	7	4	2			7.375 \pm .100	1.612 \pm .077	21.86 \pm 1.06	
Check						1	3	4	13	15	15	10	7	7	5	2	2	1				7.370 \pm .090	1.334 \pm .064	18.10 \pm 0.89	
Minus (-)						1	1	3	9	11	11	19	13	8	7	6	5	4	1	1		7.280 \pm .095	1.415 \pm .067	19.44 \pm 0.96	
Fifth period																									
Plus (+)					1	3	4	8	18	13	11	11	11	8	5	4	2	1				7.035 \pm .093	1.378 \pm .066	19.59 \pm 0.97	
Check					1	1	8	9	13	15	15	14	5	5	4	4	2	1		1		6.970 \pm .101	1.495 \pm .071	21.45 \pm 1.07	
Minus (-)					1	2	6	8	12	18	22	14	6	5	2	1	2	1				6.340 \pm .081	1.194 \pm .057	18.83 \pm 0.93	

TABLE 22

Differences in square millimeters between the means of the checks and those of the selections.

Period	Difference between means of check and plus selection	Difference between means of check and minus selection
Clone 38		
First	0.030 \pm .117	-0.075 \pm .119
Second	-0.290 \pm .137	0.240 \pm .134
Third	0.215 \pm .100	0.030 \pm .100
Fourth	0.060 \pm .113	0.210 \pm .107
Fifth	0.325 \pm .130	0.035 \pm .154
Clone 76		
First	0.095 \pm .131	0.370 \pm .139
Second	0.415 \pm .144	0.140 \pm .139
Third	0.380 \pm .121	-0.085 \pm .117
Fourth	-0.030 \pm .138	0.280 \pm .143
Fifth	0.055 \pm .135	0.035 \pm .128
Clone 79		
First	-0.060 \pm .130	0.170 \pm .131
Second	-0.010 \pm .130	0.165 \pm .124
Third	0.435 \pm .143	-0.080 \pm .138
Fourth	0.290 \pm .157	0.090 \pm .151
Fifth	0.635 \pm .166	0.175 \pm .153
Clone 81		
First	0.405 \pm .136	-0.170 \pm .127
Second	0.050 \pm .124	0.170 \pm .123
Third	0.225 \pm .123	-0.265 \pm .115
Fourth	0.005 \pm .156	0.090 \pm .147
Fifth	0.065 \pm .137	0.630 \pm .130

If the data in table 22 are examined, and if a significant difference between a selection mean and the mean of a corresponding check is assumed to be at least three times the probable error, it will be found in clone 38 that while the plus means were generally greater and the minus means generally smaller than the means of the checks, no single significant difference was obtained. In clone 76 there seems to be only one important difference, that for the plus selection in the third period. It is hard to account for this seeming effect of selection since in the last two periods of the selection, the difference was not maintained. Moreover, it will be observed that in the same (third) period, the minus series had a greater mean than the check, indicating that some factor, probably cultural, had affected the growth of the plants in the check culture, thus

rendering the opposite differences both unusual. In clone 79 there was entirely no effect of selection in the minus series. In the plus experiments, significant differences were obtained in the third and fifth periods, which may be considered as showing that selection was slightly effective. In clone 81 selection was of no avail. The single important difference obtained in the last period of the minus selection was probably due to the fact that some sort of fungous disease attacked the plants of the minus culture and the effect of the disease on size was not entirely overcome in sampling.

On the whole, it may be concluded that the results of this experiment, to shift the mean size of a clone, showed a very doubtful effect of selection without excluding the possibility that such an effect may be possible.

DISCUSSION AND CONCLUSIONS

Lemna minor is a convenient material for clonal study. It can be grown in artificial media in the laboratory, propagates fairly rapidly and, owing to its small size, it lends itself readily to extensive but well controlled observation and measurements without requiring much laboratory space. In many respects, it is comparable to *Paramecium*. One advantage it has over the latter is that one can always be sure with it that he is harvesting or sampling for measurement fully matured individuals which have therefore attained their mature size. In *Paramecium*, there is no way of determining that all the individuals being studied are absolutely fully grown. This fact subjects *Paramecium* measurement to a grave error, for in comparing the mean size of a group with that of another, the mean size is influenced by the number of immature animals, and it may readily be seen that if one of the groups propagates faster than the other, the former will have at any one time more young individuals than the latter.

While this plant can be grown in tap water alone and in tap water containing soil, the most satisfactory culture medium found, which can be controlled, is a modified PFEFFER's solution. The gradual dwindling of the plants when grown in tap water, especially when no frequent change of this is made, may be due to real lack of mineral food or to the absence of some organic growth-promoting substance which is now called an auximone. BOTTOMLEY (1917) in a recent paper found the presence of this substance essential to the normal and long-time growth of *Lemna minor* in DETMER's standard mineral solution. By placing

water extract of bacterized peat in such a solution, he was able to get continuous, luxuriant growth. Contrary to BOTTOMLEY's conclusion that *Lemna minor* cannot maintain normal growth in a mineral solution without auximones, it has been grown in this experiment in a mineral solution without the addition of any other substance. BOTTOMLEY's conclusion is unfair since he did not show that he used the other known standard mineral solutions, any one of which, as the present experiment has proved, may suit the normal growth of the plant.

The characters used as variants in this work are size and shape of frond, and the speed of budding. The length of root is a very unsatisfactory if not a useless character for this purpose on account of its characteristic and probably hereditary twisting habit.

While different strains in shape and size of frond and speed of propagation have been found to exist in a population, the number of these strains is not as large as might at first be imagined. The area of the natural habitat from which the material is collected is undoubtedly an important factor in the obtaining of a larger number of elementary strains, if such larger number exists. The smaller this area is, the more chance there is of finding the population in a high state of freedom from mechanical mixture since, owing to the rapid propagation of the plant, and under the influence of natural selection, a clone may be easily established at any one favorable spot.

Results of clonal selection to shift the mean in speed of propagation or to change the dominant shape of a clone have confirmed the pure line theory. The results of size selection, on the other hand, have not been in entire accord with JOHANNSEN's idea.

Unusual variations in shape have been observed, but they were not inherited, showing that they were merely somatic or physiological variations.

Lemna minor has been found to respond readily to different culture media. By growing it in an artificial or mineral solution, its natural size has been increased more than 100 percent and the predominating shape of a clone changed, as well as the speed of asexual reproduction hastened. Under such favorable conditions for growth, there was found greater variability in shape and size than under less favorable conditions. Moreover, acquired size, as a result of a change in growing medium, appeared to be non-heritable.

SUMMARY

1. There have been found different races in a population of *Lemna minor* as regards size and shape of frond and speed of propagation.

2. No single case of mutation has been observed in this experiment which covers a period of one and one-half years and which involved several thousands of individuals.

3. In a clone, there was found greater variability among plants grown in a mineral solution than among those grown in tap water.

4. Decreased or increased size acquired by plants through a change of cultural environment during less than a year's time was not inherited.

5. The results obtained in clonal selection either in shape of frond or in speed of propagation are in accord with the pure line theory. Selection to shift the mean size has shown slight effect in one out of ten cases.

The writer is deeply indebted to Professor C. H. MYERS not only for suggesting the subject of this investigation but also for liberal help in securing some of the apparatus and material used, as well as for valuable suggestions and criticisms throughout the experiment and the preparation for this paper. Thanks are also due to Professor H. H. LOVE for aid in some of the biometrical work and to Professor O. F. CURTIS for calling the author's attention to BOTTOMLEY's work.

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STUDIES ON INHERITANCE IN PIGEONS. III. DESCRIPTION AND LINKAGE RELATIONS OF TWO SEX-LINKED CHARACTERS¹

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INTRODUCTORY

Some years ago one of us (COLE 1912) reported that the factor producing intensity of pigmentation in pigeons is inherited in characteristic sex-linked fashion, the female being the heterogametic or heterozygous sex. At that time only a few typical examples were presented and a more complete report was promised. Since then our breeding experiments have been continued, and we are now able to present numbers which are really very considerable for such a relatively slow-breeding animal as the pigeon. In this paper we include a fuller report on this factor and a first discussion of another sex-linked color factor of peculiar interest and also give what data we have on the mutual linkage of these two. The second factor, which frequently produces a condition termed for

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convenience "dominant red," was mentioned in an earlier report (COLE 1914, p. 347). Its identification explains why investigators have presented conflicting evidence on the inheritance of red in pigeons.

Our breeding experiments with pigeons have been continuous since 1907, having been begun in that year at the RHODE ISLAND AGRICULTURAL EXPERIMENT STATION and continued there until 1910, when they were transferred to Wisconsin. The present tabulations include all pertinent data for the entire period. The junior author became associated in the work in 1911.

DESCRIPTION OF THE CHARACTERS

1. *Intensity*

The differences between the intense and the dilute series of colors in pigeons have been quite fully discussed in one of the reports mentioned (COLE 1914), and consequently need not be considered in detail at this time. The intensity factor, which we have designated *I*, acts as in mammals, with equal effectiveness on both black and red pigment. LLOYD-JONES (1915, p. 481) has shown that in the pigeon the effect of *I* is to increase the amount of pigment in the feathers, whether black or red, to approximately three times what it would be if *I* were absent. The factor affects the pigment in all parts of the plumage.²

As a consequence of the above we have in domestic pigeons the intense series of colors, black, blue and red, corresponding respectively to dun, silver and yellow of the dilute series. In most cases there is no difficulty in differentiating the two conditions, even though there are only one or two pigmented feathers in the plumage. It sometimes happens, however, that a black bird will show a lower grade of pigmentation in the juvenal plumage than after the first moult. In a few cases there may be some uncertainty as to whether a bird dying young is black or dun. In other cases the pigment in certain parts of the plumage may be altered by modifying factors so that its condition with respect to intensity is likewise uncertain, and these cases may be further complicated if the birds are nearly all white with the pigment confined to such modified regions. Practically no cases arise, however, in which the condition cannot be definitely determined by careful examination and comparison. Doubt-

² No noticeable difference has been observed in the color of the eye in the living bird, the pupil appearing jet black in those that are dilute as well as in those that are intense. In this respect the case is similar to that of mammals (see footnote, p. 186). It is possible nevertheless, that microscopic examination might reveal differences in the amount of pigment in the retina.

ful cases were checked, whenever possible, by subsequent breeding tests, and the breeding test is, of course, the only method of determining the presence or absence of *I* in birds whose plumage is entirely white.

There appears to be a rather definite correlation between amount of down on the newly hatched squab and the intensity of the definitive plumage. A complete study of this has not yet been made, but our records reveal 64 cases in which the down condition was noted and the plumage color is known. Among these there are only four positive exceptions to the rule that intense birds had "abundant" down when hatched, while on the dilute birds it was "sparse." Whether the recorded exceptions are real or due to lack of uniformity in description we are not at the present time prepared to state, for while the differences in amount of down are usually quite distinct, in other instances they appear to intergrade.

This marked correlation of sparse down with dilute pigmentation was brought to DARWIN's attention by TEGETMEIER and is mentioned by him in "Animals and plants under domestication" (1868) and elsewhere. He says:

"Mr. TEGETMEIER has informed me of a curious and inexplicable case of correlation, namely, that young pigeons of all breeds which when mature become white, yellow, silver (i.e., extremely pale blue), or dun-coloured, are born almost naked; whereas pigeons of other colours are born well-clothed with down."³

It is a matter of much interest that STRONG (1912 b) has noted a similar relation of intensity of pigmentation to abundance of natal down in the Ring-dove (*Streptopelia risoria*), the blond variety having much down while the whites have little. STRONG makes this as a general statement and does not say whether exceptions ever occur. We also have noted the same difference in Ring-doves. The matter of exceptions is of importance, since if the two conditions are really completely correlated it would indicate either two factors completely linked or else two very different physiological effects of a single factor. If true exceptions occur, it would appear that abundance of down depends upon a separate sex-linked factor, closely, but not completely linked to *I*. The fact that a

³ Reference to this observation occurs in the unpublished DARWIN-TEGETMEIER correspondence deposited in the library of the New York Botanical Gardens. It seems probable that the whites referred to were genetically dilutes; for if the correlation is between the down condition and the factor for intensity of pigmentation rather than the condition of expression of that factor, whites carrying *I* would be expected to have abundant down in the nestling stage. Our records do not permit a decision at present on this point.

parallel condition occurs in the Ring-dove suggests that the difference between the blond and white types is really an intensity difference and that the sex-linked factor which is responsible is homologous to the factor *I* in the domestic pigeon.

2. *Dominant red and gray*

That the uniform red color found, for example in the Tumbler breed of pigeons, is a simple Mendelian recessive to black was first reported by the senior author (COLE 1909) and later (COLE 1914) elaborated in more detail. The differentiating factor was given the symbol *B*. In the presence of *B*, the plumage color is black, blue, dun or silver; in the absence of *B* a uniform red or yellow.⁴

At the time the 1914 paper was being written, it had become evident that a second factor is capable of producing reddish pigmentation in pigeons, this fact being referred to on pages 326, 335 and 347 of that paper. This factor alters the expression of *B* so that at least some birds carrying both *B* and *A* are a distinct but not uniform red. Such red birds mated to homozygous blacks have red offspring, and therefore red of this type may conveniently be referred to as "dominant red" to distinguish it from the recessive red (type *ba*) mentioned above. The *A* factor, like *I*, is sex-linked in inheritance, the female never being homozygous for dominant red. The recessive red is of course inherited independently of sex.

As we at present interpret our breeding evidence, the primary characteristic of the *A* factor is its modification of black and not the production of red pigment, for we have secured a series of sex-linked gray colors, dominant to black, produced by what is apparently this same *A* factor. Whether an *A* bird shows red or gray probably depends on some unidentified factor or factors which modify the action of *A*; although the possibility that the gray-producing *A* is a third allelomorph in the set must be considered. If either of these interpretations proves to be true, no confusion will result from combining the reds and grays in discussing the *A* factor and its linkages, and this we have done in our tables. If further work should show that neither is tenable, and that the two conditions are produced by separate sex-linked factors, tables 2 and 4

⁴ To make the interpretation analogous to that now current in mammals, *B* may, if desired, be considered an extension factor for black pigmentation. In the absence of *B*, black is confined to the retina. This view may prove useful in the explanation of certain recessive red birds which bear a few dark-colored (black?) feathers, this unusual condition being interpreted as partial extension of black.

of this paper would lose some of their value and table 3 become meaningless. However, our limited evidence indicates that the two general conditions are not produced by separate factors, a point which we hope to discuss on another occasion.

Red and yellow birds carrying A but lacking B (formulas bAI and bAi) are indistinguishable in appearance from ordinary recessive reds and yellows (baI and bai). We have not found such birds among any of the recognized breeds that we have experimented with but have formed them without difficulty in recombinations. Pigeons of the dominant red type of coloration (B present) commonly show an admixture with blue of a peculiar shade usually quite distinct from the blue (which is really a light gray) seen in the familiar blue with black wing bars that occurs in various domestic varieties and in most specimens of the wild Rock pigeon. In the dominant red the relative amount of red and blue is subject to much variation, individuals occurring in which the red is very greatly reduced. The individual feathers are seldom a uniform red but show bluish towards the base where the red color runs out. The red is somewhat deeper than the color of recessive reds. The difference between the blue on these birds and that of the ordinary blue (a , with black bars) appears to be due to varying amounts of reddish pigment which occurs in most of the blue feathers, giving a somewhat "warmer" tone than that of the ordinary blue. In very clear red-barred birds this difference is not very considerable if attention is confined strictly to the clearest areas, such as the lesser wing coverts. In birds that are more red the blue takes on a distinctly brownish tinge.

A characteristic of all birds bearing both A and B is a peculiar lightening of the color of the tail that we have often in our records referred to as a "washed-out appearance." The tail is in all cases noticeably different from the body, even in birds which without A would have their pigmented areas uniformly colored. This peculiarity is frequently useful in classifying white splashes as it is the most persistent characteristic of A birds.

Some of the red and apparently all of the gray AB birds show black flecks or patches or both scattered over the body. This condition has, however, not yet been analyzed. We have seen nothing corresponding to it in non- A individuals.

Among pigeons bearing the B factor but not A , there are three general types, the uniform black, the check and the blue-barred. These have been described in some detail by COLE (1914). Each exhibits certain

variations. The uniform black may be glossy or dull. The checks show differences in the relative proportions of black and the lighter contrasting colors, while the latter in turn may be blue or a dull somewhat grayish black generally called "smoky." The barred types have two black wing bars and a black terminal tail band; the rest of the wing and the contour feathers may be a clear gray blue or a dark smoky blue. These categories are perhaps not as sharp as might be inferred from the above statements, as there are certain intergrades which may, however, for our purposes be ignored.

Both the dominant red and the gray series have types corresponding more or less closely to those of the black series. Birds of the red series that would be uniform black in the absence of *A* have wing coverts, head, neck and back of a fairly uniform red. They are light in the outer primaries, rump, tail, flanks and lower belly. These light areas are sometimes a rusty blue, sometimes a palish brown. Red checkers are readily recognized. The contrasting color on the wing coverts is either a rusty blue or a lighter brownish red, which we think correspond respectively to the blue and smoky contrasting colors in black checks. The red checks do not show a terminal tail band, the tail being approximately as in the "uniform" dominant reds. The type in the dominant red series corresponding to the blue-barred of the black series is called silver⁵ by Homer fanciers, at least in this country, but we shall call it red-barred. It has two distinct red wing bars but the general body color is bluish. This blue is sometimes very pale, sometimes dark and mixed with red. These differences doubtless correspond to the gradation from clear blue-gray to a dark smoky color found in barred birds of the black series. The tail is generally light bluish or brownish blue and faded in appearance. There is usually a deposition of brownish pigment on the under side of the tail feathers in a poorly defined area just anterior to the region that the terminal band occupies in blue-barred birds. This pigmentation may usually be seen from the dorsal surface. In the red-barred birds, then, the tail-band region commonly shows up lighter than

⁵ The term "silver" is used in other publications of this series of studies for the dilute blue, a type of coloration in which the wing bars and tail band are dun, and this is the usage of most fanciers. Those who call the birds with red bars "silvers" sometimes refer to the birds with dun bars as "silver-duns." In this paper we shall continue to use the term silver in the same sense as heretofore so as to avoid misunderstanding, since to use silver in the sense of the Homer fanciers would introduce very great inconsistencies into our terminology. "Red-barred" is the best descriptive term that has occurred to us. Adopting this however we should, to be consistent, call blue birds "black-barred" and silvers "dun-barred," but the change hardly seems necessary.

the rest of the tail. The statement made by COLE (1914, p. 326) that the terminal band is red was inexact and should be corrected.

A similar series of types presumably exists among the grays though these have not been as yet thoroughly worked out. We however have not yet secured gray checks. The most nearly uniform grays are very nearly the same shade in all parts of the plumage, even the primaries and tail being but very little lighter. As already mentioned we have not yet secured any of this type which were entirely free from black flecks, and although the red pigment may be reduced to little more than a trace in the position of the wing bars, this also appears never to be entirely absent. When more red occurs in the wing bars, red pigment appears also on other parts, particularly the head and neck, resulting in a red-barred gray corresponding to the red-barred blues.⁶

Mention has been made in the foregoing discussion, of the effects of the *A* factor only on the *intense* series of colors, red, black and blue. In other words, the presence of the intensity factor, *I*, has been assumed in all cases. What has been said of the effects of *A* applies, however, in the same way to the dilute condition of these colors. A *BA* bird lacking *I* is yellow, just as an ordinary red bird (*ab*) is yellow if *I* is absent, and the dilute condition of the bird with red wing bars (*BsAI*) is similarly a modified bluish or grayish, with yellow instead of red wing bars.

The *A* factor is undoubtedly of wide distribution in different breeds of pigeons. By breeding tests we have identified it in Homers, in white clean-legged Tumblers, in white-muffed Tumblers, in white Fantails and in red-and-white Tumblers showing the Baldhead pattern, all of which were secured from widely different sources. It was apparently present in many of the birds which BONHOTE and SMALLEY (1911) used in their experiments. The birds referred to by them as "dark mealy," "light mealy," and "white mealy," all of which are figured on plate XXV accompanying their paper, obviously carry the *A* factor, the first-mentioned either being identical with, or at least resembling very closely, what we have termed red-barred. On the other hand, their "blue," "silver" and "chequer," figured on plate XXIII, correspond to the types for which we use the same terms, and as obviously do not carry *A*. Furthermore the birds they designate as "grizzles" (*loc. cit.*, plate XXIV) are apparently modified *a* types, a fact which is rendered more conclusive by a comparison of the grizzle and mealy feathers depicted on plate XXVI. This interpretation

⁶ Descriptions in some detail of specimens selected to represent several of the dominant red and gray types will be found as an Appendix to this paper, p. 202.

accords with their statement that mealy is dominant to grizzle and, in the light of our own investigations on the *A* factor, helps to clear up their difficulty in explaining "the predominance of one sex in certain colours." In a footnote on p. 617 they say:

"This most interesting question has not been dealt with in the present paper, as we have not yet fully investigated the results; but we may mention that a large proportion of the White Grizzles are ♀'s, and in the Light Mealies by far the larger number are ♂'s; we have also bred a certain number of Cream Mealies, and these have all been ♀'s."

If BONHOTE and SMALLEY had tabulated their results by individual matings they doubtless would have found that in certain matings all the males were mealies and all the females grizzles, since this would be the expectation whenever grizzle cock was mated to mealy hen. Their last statement, that all the "cream mealies" bred were females, is difficult of explanation, since by hypothesis, if these birds also carried the *A* factor, they would be expected to be males if there were any restriction as to sex. We are not certain, however, just what the authors mean by "cream" mealies, since they do not appear to be described or mentioned elsewhere in the paper.

Evidence that the *A* factor was involved in STAPLES-BROWNE'S experiments on inheritance of color in pigeons is not so definite, although his statements (STAPLES-BROWNE 1908, p. 70) that possibly "two types of reds may eventually be demonstrated," and (STAPLES-BROWNE 1912, p. 133) that the question of the inheritance of red and yellow is a complicated one, may have been induced by complications due to the presence of this factor in some of his birds. Among other breeds he employed Fantails in his experiments, and, as already stated, we have identified the *A* factor in individuals of this breed.

A series of experiments recently reported by NUTTALL (1918) also involves this same factor. Nuttall worked with the "Racing Pigeon," which is probably the same as our Racing Homer. He divides his birds into four color varieties, viz., blue, blue checker, mealy and red checker. He finds red (of the red checker or mealy) dominant. His use of blue is the same as ours, namely blue birds with black wing bars and tail band; blue checkers are blues plus checking. Regarding the mealies and red-checkers he states:

"The colour of the so-called mealy birds is difficult to describe. The ground colour is somewhat like that of fine oat-meal; the wing-bars are reddish—approaching the colour of damp sand. The mealy birds differ in two salient points from the blues—the wing- and tail-quills are, as a rule, pale in colour, and the tail-bar is absent.

"The red-chequered birds stand in the same relation to the mealies as the blue chequers do to the blues, i.e., they are mealies with the addition of chequering. The wing-quills and tail-quills are generally pale in colour, there is no tail bar."

NUTTALL finds the red (of the red checker or mealy) dominant to blue, and check dominant to its absence,⁷ and assumes two sets of allelomorphs to account for the four color types. These are: *R*, presence of red; *r*, absence of red; *C*, presence of checking; and *c*, absence of checking. The types may be represented by phenotypic formulae as follows: *RC*, red checker; *Rc*, mealy; *rC*, blue checker, *rc*, blue. On the strength of the descriptions quoted above and the behavior of red in relation to black (of the blues) in the breeding tests, there can exist no doubt whatever that NUTTALL's red checker is the same as our dominant red check, and that his mealy corresponds to the mealies of BONHOTE and SMALLEY (1911) or what we have termed red-barred. NUTTALL's factor *R* is therefore identical to our *A*, but owing to the fact that he lumped his data and did not consider the results of individual matings separately, he apparently gained no inkling of its sex-linked nature.⁸

EXPERIMENTAL RESULTS

1. *Inheritance of the I factor*

In the case of a sex-linked factor, only three types of matings provide evidence of segregation. For the *I* factor, these are:

1. ♀ *I*- × ♂ *Ii*; expectation, 2 intense ♂♂ to 1 intense ♀ to 1 dilute ♀.
2. ♀ *i*- × ♂ *Ii*; expectation, 1 intense ♂ to dilute ♂ to 1 intense ♀ to 1 dilute ♀.
3. ♀ *I*- × ♂ *ii*; expectation, 1 intense ♂ to 1 dilute ♀.

Table 1 gives a summary of the results obtained from all three types of matings. This covers the offspring of 78 matings or families of type 1, 70 families of type 2, and 60 families of type 3. A detailed presentation of the results from individual matings is omitted in the

⁷ The dominance of check was first mentioned by BATESON in 1909 on the authority of STAPLES-BROWNE, and later verified by BONHOTE and SMALLEY (1911) and by COLE (1914, p. 335).

⁸ NUTTALL summarizes his results according to phenotypes crossed and assumes that the possible genotypes in each phenotype are present in equal proportions—in itself a dangerous assumption with such small numbers. Unfortunately, furthermore, in considering the possible genotypes of red checker he makes an error (considering *RCrc* and *RcrC* as different) which vitiates his calculations throughout. Consequently, while his expected appearances are correct as to classes, they are faulty with respect to numbers.

present report as it would occupy considerable space and would serve little purpose. It is to be understood that all matings meeting the requirements and showing segregation with respect to intensity have been included, whatever the color. In some cases the *A* factor is involved, and in many the birds may have been entirely white except for a few feathers by which the intensity could be determined; or in some instances they were entirely white and the question as to whether they carried *I* was determined from breeding results.

The first thing to be noted from this table is that with one possible exception all records fall into the classes which would be expected from the nature of the respective matings.⁹ The doubtful case is a single dilute male recorded in type 1. As a matter of fact the descriptions of this bird show uncertainty, for it was described on September 15, 1908, when somewhat less than a month old, as "apparently a good white bird except for flecks of *red* at the tips of some of the primaries, secondaries and greater primary coverts." After molting, however, it was again described on January 23, 1909, as "pure white except for a very little *yellow* at the tip of one greater primary covert, in secondary of right wing, and in one primary of left wing." This individual was included as dilute in the earlier report on the basis of the later description, but it now seems extremely probable that the first description is the correct one. On account of the uncertainty, however, the bird is not included at all in the present computations. It is clear, considering the contradictory descriptions, that it should not be counted as an exception to the sex-linked segregation of *I*. We thus find for the intensity factor in pigeons no cases of "partial sex-linkage" such as those reported in the Ring-dove

⁹ There are two matings, not included in the table, in which dilute birds appeared where they were not expected at all. A white female (364A) with only a few dark-pigmented feathers was bred to a homozygous black male, and among the offspring was a female (687B) which was white except for a few *yellow* feathers. She in turn was bred to a black male homozygous for intensity, and among their offspring also there was a white bird, this time a male (909A), with a few yellow feathers. We have noted these birds as "aberrant yellows" and as yet have no explanation of the case. It is complicated by the fact that the birds were so largely white, and it is known, furthermore, that some of them carried the *A* factor, which complicated matters still further. It should perhaps be mentioned furthermore that these early results were obtained at a time when the matings were not controlled as closely as they have been in the later years of the work. Until 1911 the mated pairs were not isolated but were kept together in large pens, though special care was taken to prevent cross-mating and to detect it if it occurred (COLE 1914, p. 318). Beginning in 1912 the use of separate pens for pedigreed matings was begun, and this practice was extended so that there were very few non-isolated pairs in 1914 and all matings have been strictly controlled since that time.

TABLE I
Inheritance of I.

Type of mating	$\delta \delta$		q q		Sex?		Color?		Total, color		Total, sex		Sex ratio
	I	i	I	i	I	i	$\delta \delta$	q q	I	i	$\delta \delta$	q q	
1. δIi — q I-	Obtained	220	(1)	88	20	5	79	61	330	93	300	239	125
	Expected	199 204	—	99.5 97	19 19	6 6	70 72	70 68	317 320	106 103	269.5 276	269.5 263	100 105
2. δIi — q i-	Obtained	106	73	87	85	11	94	91	204	170	273	263	104
	Expected	87.5 90	87.5 90	87.5 85.5	11.5 11.5	11.5 11.5	92.5 95	92.5 90	186.5 192	186.5 182	268 275	268 261	100 105
3. δii — q I-	Obtained	124	—	—	115	14	37	31	137	129	161	146	110
	Expected	119.5 122	—	—	119.5 117	13.5 13.5	34 35	34 33	133 136	133 130	153.5 157	153.5 150	100 105
Total sexes	Obtained	—	—	—	—	—	—	—	—	—	734	618	113
	Expected	—	—	—	—	—	—	—	—	—	691 708	691 674	100 105

by STAPLES-BROWNE (1912) and STRONG (1912 b).¹⁰ The attempt was made by BRIDGES (1913) to explain these exceptions on the assumption of a pair of sex chromosomes in the female dove and a discrete "sex-differentiating factor" located near the factor for plumage color. Later, however, he puts forward the opinion that "non-disjunction offers an alternative explanation which seems more plausible" (BRIDGES 1916, p. 157). We are inclined to believe that the records are more probably in error.

Even a casual examination of the table reveals an almost consistent excess of males and of intense birds beyond expectation; only in a few instances is this not true, and then the differences are very slight. An excess of intense birds was noted and commented upon at the time of the earlier report on the segregation of the *I* factor (COLE 1914, p. 360). In that report sex was not considered. It was then suggested that the discrepancy between observed and expected ratios might have been due in part at least to errors in determination of the color in some instances. This explanation has now, however, been definitely ruled out, since it has been found that such errors were at most exceedingly infrequent in the earlier records, and there is even less probability of their occurrence since that time. Nevertheless, although the total number of cases has been more than doubled since reported in 1914, the excess of intense birds is in general even greater in proportion than it was then.

The fact that there is an excess both of males and of intense birds, suggests at once a causal relation between the two, especially as the intensity factor is sex-linked. It seems very probable, in fact, that this is the true explanation of the excess with respect to intensity. It does not, however, explain why the males should be so much in excess in many cases. If the normal ratio of males to females in a heterogeneous population of pigeons be accepted as 105:100 (COLE and KIRKPATRICK 1915, p. 465),¹¹ it will be seen that in the matings of

¹⁰ In table II of STRONG's paper (l.c., p. 301) and on page 313 he states that he obtained three blond females from mating of white male to blond female, from which only blond male and white female offspring would be expected. Tables XXI and XXII, however, which present his results in detail, show only two recorded exceptions (in mating 29) and this is the number he himself states he obtained in another paper (STRONG 1912 a, p. 443). This may perhaps cast some doubt on the validity of the two recorded exceptions.

¹¹ As stated in a footnote on the page referred to, a count of all sexes determined to December 1, 1914, gave 919 males to 881 females, a ratio of 104.31:100. A tabulation of all sexes recorded up to January 1, 1918, shows 1632 males to 1537 females, or a ratio of 106.18:100. This again indicates that the average ratio for our population tends to vacillate around 105.

type 2 the results came fairly close to this norm; but in the other two types of matings the ratio was much higher. Particularly is this so for type 1, where the males to females are as 125:100. The numbers on which this ratio is based are too large for this to be a chance deviation.

In presenting for comparison the expected results with those actually obtained, the expectations are given based both on normal, unmodified Mendelian segregation of sex (ratio 100:100) and of the factor for plumage intensity and also on the assumption that the average sex ratio is for some unknown reason 105:100. It will be observed that the latter figures tend to correct in considerable degree the discrepancies which occur in the case of the former. Even so, however, a decided excess still remains to be explained. We are unable at present to suggest any well substantiated explanation, but a careful study of the individual matings, particularly of type 1, where the excess noted is greatest, brings out certain facts which are suggestive. These are not definite enough to warrant the inclusion of the detailed data here, but it is hoped by further breeding in selected families to secure large enough numbers for more adequate analysis. The most significant fact apparent is that the great excess of males, and consequently of intense individuals, appears to be due mostly to widely divergent ratios in certain families and not to a general tendency in all. If we were to assume a sex-linked lethal factor in such families, closely associated with *I*, the excess of male offspring could be accounted for. The ratio of males to females in lines carrying the lethal should be 2:1, and in a few matings where there is a fair number of offspring the proportions approximate this ratio. The closer the association of *I* with the lethal factor, the nearer would the ratio of intense to dilute offspring also approach that of 2:1. The proportions of the sexes and of intense and dilute birds in any population would accordingly depend upon the relative number of lines carrying the lethal and the closeness of association between the lethal and the factor *I*. While this hypothesis is admittedly hung on a very slender thread of data, it at least has the advantage that it can be tested by definite and known experimental methods.

2. Inheritance of the A factor

In table 2 are shown the data on the inheritance of the *A* factor. Here again it will be noted that all fall in the expected categories, with two possible exceptions,¹² a recessive (*a*) male and a dominant (*A*) female,

¹² An exception not included in the table occurred in the case of ♀ 687B, one of

in one of the matings of type 3. The records themselves in this case contain no indications of confusion, but it seems significant that the two birds in question were nestmates, and that if the sexes were simply reversed, they would fall into their proper classification.¹⁸ This happens, furthermore, to be one of the relatively few instances in the earlier part of the work in which the recording of the sex was done by an assistant in the absence of the senior author, and before the junior author was associated with the work. All things considered, the probability of a reversal of the sexes having been made in recording seems strong enough to warrant these birds not being counted as exceptions; instead, they have simply been omitted from the computations altogether.

There is a striking difference in the sex ratios in table 2 as compared with table 1. In table 1 there was an excess of males in all three types of matings, as has been seen. In table 2 just the opposite is the case in the first two types of matings, while in the third there happens to be exact equality. While in most cases the observed numbers are fairly close to expectation on a basis of equality of males and females, they are still farther away from expectation on a basis of 105:100. The very low ratio of males in type 1 cannot be given great weight because of the relatively small numbers, but larger numbers are involved in the other matings, and there can be no doubt that the general deficiency of males in table 2 as compared with table 1 is significant. The theory suggested to explain the excess of males in the former instance cannot be used to

the "aberrant yellows" already referred to in the footnote on p. 192. The recorded male parent of this bird was a black (*aa*) and the mother (presumably *A*—) was white with a few dark-pigmented (dominant red?) feathers. In such a mating none of the female offspring should carry *A*, but ♀ 687B evidently did. Two other birds (1533B₂ and 1608V) having the *A* factor are recorded from parents neither of which had it. These birds also are related, the father of 1608V being a brother of 1533B₂, a fact which may have significance. Since, however, these cases are so irregular, and as no explanation is at present apparent, they have been omitted from consideration in the present connection. Cross mating or confusion of records is possible, but we have found nothing except the discordant results to indicate that anything of this kind is responsible. The later cases are being continued for further investigation.

¹⁸ In this connection it might also be mentioned that in the early work at Rhode Island the letters A and B were used to designate the squabs hatched from the first and second eggs respectively, the records being intended for use in a study of the relation of order of laying of the eggs to the sex of the resulting offspring (COLE and KIRKPATRICK 1915). When the identity of egg and squab was lost the symbols X and Y were used to indicate that fact. It is possible that this may have some bearing on the apparent reversal of the sex records in the present case, for the nestmates in question were an X and Y pair and the assistant who made the record of sexes, knowing that they would be useless in that investigation, might not have made the entries with his usual care.

TABLE 2
Inheritance of A.

Inheritance of *A*.

Type of mating	$\delta \delta$		q q		Sex?		Color?		Total, color		Total, sex		Sex ratio $\delta \delta: 100 \text{ q q}$
	<i>A</i>		<i>a</i>		<i>A</i>		<i>a</i>		<i>A</i>		<i>a</i>		
	<i>A</i>	<i>a</i>	<i>A</i>	<i>a</i>	<i>A</i>	<i>a</i>	$\delta \delta$	q q	$\delta \delta$	<i>a</i>	$\delta \delta$	q q	
1. δAa	14	—	8	8	6	1	6	13	28	9	20	29	69
q A-	15	—	7.5	7.5	3.5	3.5	9.5	9.5	28	9	24.5	24.5	100
Expected	15	—	7.5	7.5	4	3	10	9	28	9	25	24	105
2. δAa	57	48	53	49	4	6	34	41	114	103	139	143	97
q a-	51.75	51.75	51.75	51.75	5	5	37.5	37.5	108.5	108.5	141	141	100
Expected	53	53	50.5	50.5	5	5	38	37	111	106	144	138	105
3. δaa	65	(1)	(1)	64	1	4	27	28	67	69	93	93	100
q A-	64.5	—	—	64.5	2.5	2.5	27.5	27.5	68	68	93	93	100
Expected	66	—	—	63	3	2	28	27	70	66	95	91	105
Total sexes	—	—	—	—	—	—	—	—	—	—	252	265	95
Expected	—	—	—	—	—	—	—	—	—	—	258.5	258.5	100
	—	—	—	—	—	—	—	—	—	—	265	252	105

explain the deficiency of them here; but if the suggested lethal were entirely absent the ratio of males should not be higher than 100. If the two sets of data are combined, duplicates being counted only once, thus making up a larger sample of the total population of the lofts, the total numbers are 898 males to 832 females, or a ratio of 108:100. This number is fairly close to 105, which has been taken as the average of the general population. It would doubtless have approximated it even more nearly had the number of birds involving the *A* factor been as large as that included under *I*.

The foregoing illustrates how general population averages are derived from composites and in themselves may have little if any direct biological significance.

3. *Linkage relations of I and A*

Since *I* and *A* are both sex-linked in their inheritance, it is naturally to be expected that they should show some linkage to each other. We have been endeavoring for the past four seasons, by experiments designed definitely for that purpose, to obtain sufficient data to establish such linkage beyond reasonable doubt and to obtain a reliable measure of its intensity. GOODALE (1917) has recently reported crossing over of sex-linked factors in the fowl, where it occurs in the male as is the case in Lepidoptera. This is apparently the first published record for birds. We have been aware of crossing over in the male pigeon ever since the second sex-linked character was recognized in 1913; our difficulty has been to demonstrate that there was any linkage. Only relatively few matings could be devoted to this purpose, and reproduction in pigeons is so slow that our numbers are still inadequate to give anything like an exact measure of crossing over. While, therefore, we do not claim that the figure obtained to date indicates the precise amount of crossing over between these two factors, we do feel nevertheless that the divergence from equality of crossover and non-crossover types among the offspring is sufficient to indicate that an appreciable, though probably relatively slight, degree of linkage exists.

In table 3 the details are given of those matings used in the linkage computations. In the upper part of the table are matings of males who received both dominant factors from one of their parents and both recessives from the other. The combination given first is that received from the father and the second is that from the mother. Thus ♂ 896B in mating 1320 received the recessive factors *ia* from his father and the dominant factors *IA* from his mother; and so for the others. The results

TABLE 3
Matings showing linkage of I and A.

Number	Mating				Offspring			
	♂ parent		♀ parent		Non-crossover		Crossover	
	Number	Formula	Number	Formula	<i>I</i> <i>A</i>	<i>i</i> <i>a</i>	<i>I</i> <i>a</i>	<i>i</i> <i>A</i>
1320	896 B	<i>ia.IA</i>	1028 B	<i>ia—</i>	0	2	0	1
1329 ¹⁴	711 A	<i>ia.IA</i>	655 A	<i>IA—</i>	0	1	0	0
1450	896 B	<i>ia.IA</i>	450 Y	<i>ia—</i>	2	0	0	0
1511	1470 B	<i>ia.IA</i>	847 A	<i>ia—</i>	5	4	3	2
1512	1457 B	<i>IA.ia</i>	1332 B	<i>ia—</i>	4	0	2	0
1580	1061 H	<i>IA.ia</i>	781 B	<i>ia—</i>	2	2	0	1
1602	1457 B	<i>IA.ia</i>	1304 K	<i>ia—</i>	6	1	3	4
1617	1457 F	<i>IA.ia</i>	1325 C	<i>ia—</i>	1	1	2	1
1620	1061 H	<i>IA.ia</i>	1471 E	<i>ia—</i>	1	0	0	0
1624	1511 E	<i>IA.ia</i>	1470 K	<i>ia—</i>	1	1	0	0
Totals					22	12	10	9
					<i>I</i> <i>a</i>	<i>i</i> <i>A</i>	<i>I</i> <i>A</i>	<i>i</i> <i>a</i>
1365	968 B	<i>Ia.iA</i>	903 A	<i>ia—</i>	1	0	2	0
1456 ¹⁴	1167 A	<i>iA.Ia</i>	1432 A	<i>Ia—</i>	2	1	1	1
1523	1335 M	<i>iA.Ia</i>	781 B	<i>ia—</i>	5	1	3	3
Totals					8	2	6	4

Summary: 44 non-crossovers, 29 crossovers.

Percent crossing over, 39.72.

of these matings tend, therefore, to show "coupling." The three males in the lower part of the table received one dominant and one recessive factor from each parent, and their offspring accordingly indicate a corresponding "repulsion" of *I* and *A*. Of the 73 offspring obtained in these 13 matings, 29 were crossovers and 44 non-crossovers. Thus crossing over occurred in 39.72 percent of the cases. Although with such small numbers a few records one way or the other may change the percentage value considerably, it has been noted as the records have accumulated that this value has tended to remain somewhere near 40 percent. For the present, therefore, until larger numbers have been obtained, it may be assumed that the crossover value of *I* and *A* is roughly 40 percent.

No special demonstration of the absence of crossing over in the female is necessary, since it is sufficiently demonstrated in the matings recorded in tables 1, 2 and 3. If crossing over were to occur in the female it would result in exceptions to the expected relations of sex and the sex-

¹⁴ Only female offspring considered.

linked characters, but no such undoubted exceptions were found. Furthermore, crossing over in the female would make possible the occurrence of females homozygous for the sex-linked characters, and no such have ever appeared. The reported cases of exceptions in the dove and canary are doubtful, and if valid, are probably to be accounted for on another basis (BRIDGES 1916). GOODALE (1917) reports no crossing over in the female domestic fowl, so it seems legitimate to assume that it does not occur in birds. This means that if the sex chromosome of birds has a mate, as in *Drosophila* it does not bear any factors.

The best test of crossing over in the female is obtained when males recessive for both the sex-linked characters (*ia.ia*) are mated to females carrying them both (*IA—*). Under these conditions all the male offspring should carry both *I* and *A*, while the female progeny should be double recessive (*ia*). In our records there are three matings of this type. These are shown in table 4, and as far as they go they show results entirely in accord with the expectations on the assumption that crossing over does not take place in the female.

TABLE 4
Demonstrating absence of crossing over in the female.

Mating					Offspring		
Number	♂ parent		♀ parent		Non-crossover		Crossover
	Number	Formula	Number	Formula	♂ <i>IA</i>	♀ <i>ia</i>	
248	38 A	<i>ia.ia</i>	55 A	<i>IA—</i>	0	2	0
830	697 B	<i>ia.ia</i>	548 A	<i>IA—</i>	2	2	0
1470	1285 U	<i>ia.ia</i>	1333 A	<i>IA—</i>	7	8	0
Totals					9	12	0

Crossing over, 0 percent.

SUMMARY

1. Two sex-linked characters of the domestic pigeon have been studied, namely intensity of pigmentation (factor *I*), and an alteration in the appearance of the black pigment (factor *A*).

2. The *A* factor has a variable effect on the color of the bird, the differences depending, presumably, upon combinations of individual factors. There are apparently two main categories, *dominant red* and *gray*. The dominant red presents an interesting contrast with the *recessive red* described in previous publications.

3. In the case of *I*, while the results were in accord with expectation as to the association of the character with sex, there was a considerable disturbance of the sex ratio, the males being much in excess of expectation. This seems to be due largely to excess of males in particular families, and may be the result of a recessive sex-linked lethal factor.

4. In the matings involving the *A* factor there was a deficiency rather than an excess of males. No explanation is apparent.

5. The two sex-linked factors *I* and *A* show a slight but appreciable mutual linkage. Crossing over in the male occurs in roughly 40 percent of the cases; there is no crossing over in the female.

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APPENDIX

Descriptions of dominant red and gray types

The following descriptions of individual birds selected to represent the more striking types of dominant reds and grays may be of value to the reader who desires to get a more definite notion of the colors referred to as resulting from the action of the *A* factor. Only brief and relatively incomplete descriptions are given at this time as it is planned to present much more detailed descriptions of these types after the genetic relations of the various conditions have been more thoroughly worked out. The color names in quotation marks are those given by RIDGWAY (1912).

1533B₂. *Dominant red, light rump*. Head, neck, breast, upper back and wings (except flights), dark reddish brown. Feathers taken from lesser wing coverts are slightly lighter than "Vandyke brown"—between that and "Rood's brown," but nearer the former.¹⁵ On the head there is a slightly bluish cast, and the same is true on the lower breast and belly. Flights (except at tips) and primary coverts mealy (red and blue mixture). Tips of flights, rump and tail light steely blue ("gull gray"). In these parts there are prominent flecks of black.

1487B₂. *Dominant red, dark rump*. Distribution of red as in previous type; darker in color, apparently on account of numerous fine intermingled specks of black. Lesser wing coverts match very closely with RIDGWAY's "bister." Lower breast and belly very dark, almost black. Flights slaty (with reddish cast), not conspicuously lighter at tips. Rump and tail slaty, without the reddish. Rump a dark gray difficult to match, but somewhere near "dark mouse gray." Patches of black occur in upper tail coverts and tail, but not so many small flecks as in former case.

A80. *Red barred, clear*. Head, neck, upper breast and wing bars red. On the darkest part of the breast this is close to "burnt umber"; on the tertiaries, where it forms part of the wing bars, it is lighter, about "Vandyke brown." The proximal parts of the flights and their greater coverts are mealy. These mealy areas are continuous with the wing bars when the wing is open. Most other parts of the bird are a light bluish with a slightly reddish cast, the lesser wing coverts matching fairly well with "pallid mouse gray." The rump is lighter, almost white. Black flecking practically absent.

A7 shows some modification from this type. The distribution of red is less extensive, the head being a slaty blue (near "light neutral gray")

¹⁵ The color of recessive reds (*ba* or *bA*) is lighter, commonly very close to "pecan brown" (COLE 1914, p. 320).

and practically free of red. The red of the breast is of about the same shade as in A80, but that of the wing bars is more brownish, the red bar on the tertiaries being close to "mass brown." Very little of the mealiness in the flights. The blue parts differ in lacking the warm tone of the other, owing to much less of the reddish cast mentioned. The color of the lesser wing coverts is nearly the same as is common on ordinary blues with black bars, namely "gull gray." Rump almost white. Black flecks and patches moderate in amount.

1456A. *Gray*. Fairly uniform gray, but darker on head, neck and breast, due in part to minute reddish specks in the feathers. The dark breast feathers are fairly near to "dark mouse gray" (not considering the inconspicuous brownish flecks). Lesser wing coverts "pale neutral gray." Incomplete reddish wing bars due to red-mealy patches in tertiaries and inner secondaries. Rump nearly uniform with back and tail. Numerous conspicuous black flecks and patches on nearly all parts.

A BIOMETRICAL STUDY OF CROSSING OVER. ON THE MECHANISM OF CROSSING OVER IN THE THIRD CHROMOSOME OF *DROSOPHILA* *MELANOGASTER*¹

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INTRODUCTION

This paper is an attempt to analyze the current theories of crossing over by a study of its normal fluctuating variations in a particular chromosome. It is the direct outgrowth from, and indeed in some respects, a supplement to the studies of crossing over made during the past few years in this laboratory. During this investigation, however, the problem has been approached from a different point of view and has made use of rather more adequate methods.

The problem and the point of view taken may be best defined by considering a few of the already known facts concerning crossing over in *Drosophila*. If one counts separately the offspring of a large number of back-crossed females heterozygous for a large number of factors, there

¹ A contribution from the Zoölogical Laboratory of COLUMBIA UNIVERSITY.

may be formed from the resulting data characteristic curves of variation of the number of breaks for each region of the chromosomes which were contained in the females under consideration. The precise form of these curves, as well as the location in them of the value calculated from the offspring of any particular female, is the result of two basic variables, environment and heredity;—environment, in that the conditions surrounding the germ cells of one female may be more favorable to crossing over in one region or mayhap in the whole chromosome than the conditions in another female; heredity, in that a gene may, when substituted for another gene in the same locus, influenced in a marked way the crossing over in a given region.

Little has been done toward a direct analysis of factors such as these in their bearing on the contending views of the mechanism behind the crossing-over phenomena. Yet clearly such an analysis offers one of the best means of extending our knowledge and furnishes critical evidence. The study of interindividual variation offers a way by which the problem of the mechanics of crossing over may be attacked.

Specifically, the direction of the attack on the general problem is that of the analysis of the variation curve in terms of its component individuals. A given individual in the frequency distribution may show a particularly high rate of crossing over for one section of the chromosome. Will it show the same high rate for other sections and, if so, will it also show this proportionately high rate for the double crossing over including these two regions? Does the substitution of other genes for those normally present affect the crossing over in an individual concerned in the formation of our variation curve? These examples will give a definite idea as to the general manner of approach to the problem of variation in crossing over followed in this paper.

MATERIAL AND METHODS

Most of the data contained in this paper were collected during the years 1915-'16 and 1916-'17 that the author has been a member of the Zoölogical Laboratory at COLUMBIA, the rest was obtained at Cold Spring Harbor, Long Island, during the summer of 1916.

To make the conditions as nearly constant as possible with regard to temperature, all flies were bred and reared in an incubator controlled by a thermostat to maintain a temperature of 25° C. Even with this precaution, it is realized that this is not altogether satisfactory, for in summer the outside temperature often rises higher than 25° C. However this rise is slight, and it is thought that the conditions have been

maintained so constant that temperature variations may be said to be negligible.

It is probable that food has no effect on crossing over. But as a change was made from fermented banana to an artificial food mixture of starch, sugar, peptone, yeast, and water, this factor will be discussed in connection with the data.

The factors used throughout were those which lie in the third chromosome. Enumerated in the order of their position, they are sepia (s_e), Dichaete (D'), curled (c_u), peach (p^p), spineless (s_s), hairless (H'), sooty (c^s), and rough (r_o). These factors are arranged in the chromosome as seen in diagram 1.

Several distinct sets of experiments were made, using different combinations of these factors. In all cases they were made as back-crosses of a heterozygous female to a male homozygous for the recessives carried by the female. The specific kind of cross that was used in each experiment will be given in connection with a discussion of the data.

For the data on the effect of selection on crossing over, all of the matings were made strictly brother and sister. For the rest of the data this practice has not always been followed, although it generally has been. In all cases the record for the output from each female, represents the offspring of that female mated to a single male. The time allotted for the hatching of the eggs which were laid is in every case ten days after the first fly hatches. Thus the count of a given female is obtained by counting all flies which hatch during the ten days following the emerging of the first offspring from the pupa case.

It hardly seems necessary to say that contamination was carefully watched as a source of error in the data. In every case all of the triple crossovers were tested to be sure that there were no mistakes. Any other cultures which gave extreme results were bred from to test the result. From this it is thought that the cultures included in these data are free from contamination and non-virgidity errors.

In recording data, each region of the chromosome may be designated

in one of two ways. Thus $\overbrace{s_e}^1 \overbrace{D'}^2 \overbrace{s_s}^3 \overbrace{c^s}^4 \overbrace{r_o}^4$ the first region would be either $s_e D'$ or 1; the second region $D' s_s$ or 2, etc. In this way the regions are designated from left to right numerically as 1, 2, 3, 4, etc. The double crossover may also be recorded as $s_e D'$ and $D' s_s$ where a break occurs in the two regions sepia Dichaete and Dichaete spineless simultaneously, or it may be recorded as 1, 2 (break in region 1 and break

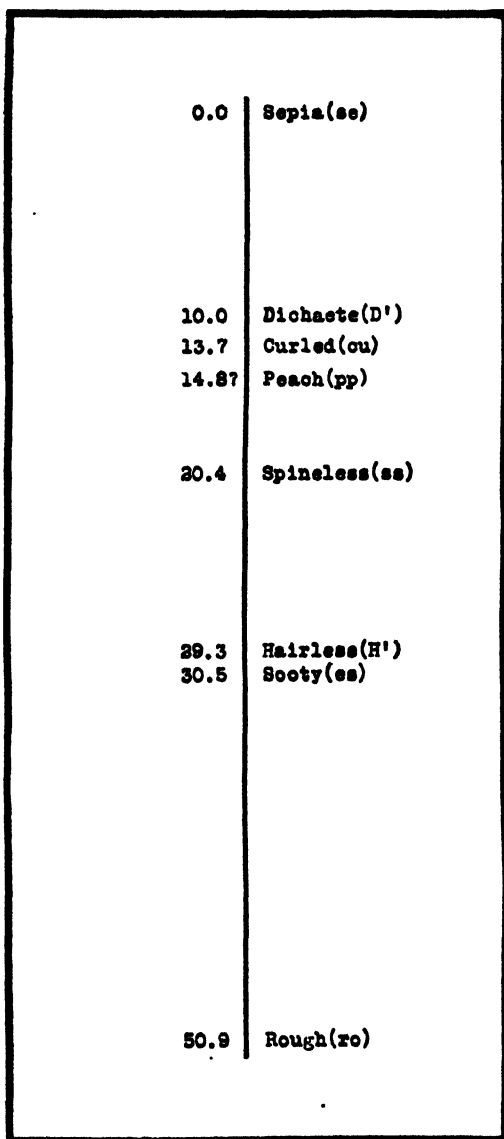


DIAGRAM I

in region 2). The triple crossovers may likewise be recorded as 1, 2, 3, for one including the first three regions of the chromosome. Thus by an extension of this method all possible crossovers are recorded.

The biometrical methods used in the analysis of the statistical material are in general not different from those commonly in use. A few remarks may not be out of place, however, in regard to the computation

of some of the constants in this paper. In the calculation of the standard deviation SHEPPARD'S correction for the second moment was not used as it is evident to anyone studying the distribution that there is no approach to high contact at one end of the distribution at least. In the back-cross test of heterozygous females, carrying the genes for sepia, curled, spineless, sooty, rough, in one chromosome, and for dichaete, and hairless in the other, by males homozygous for sepia, curled, spineless, sooty, and rough, all of the calculations were made from ungrouped frequency distributions. In the formation of the correlation tables all the data have been punched on cards and sorted, first into the frequency distribution for the first region, then the classes sorted into the frequencies for the second region to form the correlation surfaces. This sorting has all been done by the machine made by the Tabulating Machine Company. All of the calculation was done on one of the common calculating machines. It is hoped that there are no errors remaining in the computations, although it is impossible to be absolutely sure in a work as large as this that slight arithmetical slips have not gone by unnoticed.

THEORETICAL ASPECTS OF THE PROBLEM OF THE MECHANISM OF CROSSING OVER

Before proceeding to the direct analysis of the problem of crossing over, it may be well to consider what the different theories of crossing over should give as observed results in a theoretically perfect experiment. The theories to account for crossing over which are now extant may be reduced to two.² The first of these, brought forward by BATESON and PUNNETT, as the reduplication theory, attempts to account for crossing over as a differential rate of division in germ-cell formation. The second takes as its fundamental postulate a twisting of the chromosome threads in loose twists.

If we carry the analysis of what would be expected on the reduplication theory to include, besides the single separation, those double separations of coupled factors, we would expect only such correlation between single separations and the successive double separations as would be brought about by their being correlated to the same thing. In other words, we would expect this relationship to vanish when we used partial correlations to measure directly the single separations and double

² In view of the recent criticism by STURTEVANT and BRIDGES of the hypothesis to account for crossing over brought forward by GOLDSCHMIDT, it seems to me wise to await the reply before considering it further.

separations. This is just what we would not expect on the twisting hypothesis of crossing over. Let us consider the case of a fixed point of twist having a known variation around a mean ratio (a) from the fixed point for the second twist. Now, if we take successive ratios of crossing over along this chromosome, with a break at one fixed point, what is the likelihood of another simultaneous break in successive regions as we progress along the chromosome away from the fixed point? Surely, it will increase to the mode of our frequency curve of the ratio for twist, and diminish from that toward the further end. Thus one of the strongest pieces of evidence that can be given for the twisting hypothesis to account for crossing over will be given if we can show that there is such a rise and fall in single and double correlations.

There are, unfortunately, some difficulties in our data which should be pointed out. In the first place, it is impossible to limit our first break to a fixed point because there are not enough good factors close enough together to do this. It is necessary, therefore, to take a small segment of the chromosome from which to measure. The successive regions taken to divide the frequency distribution of twisting have to be uneven intervals and further the interval in one of our segments ($c^s r_o$) has to be quite long. These are physical difficulties which I see no way of overcoming. They are not difficulties which in any way vitiate the conclusions, however, for in every case the effect is such that it subtracts from the numerical value of the coefficients measuring the relationship. Thus conclusions drawn from these coefficients have a big margin of safety.

These difficulties should, however, be kept constantly in mind in weighing the value of the evidence. Specifically, in our data, region $c^s r_o$ is poorly suited to this study because it is so long that should twists occur between 25 and 30 units apart the second twist might fall in either of two regions ($s_s D'$ or D'_s), depending on whether the first one is near c_s or r_o . The mid-regions are also not well suited to this study as the regions on either side are not long enough to enable the mode of the curve to appear, if the modal frequency of twisting is about 25 units. The $s_s D'$ region considered in connection with the rest of the data is, however, well suited to the study, for here the first region is short and so located at the end of the chromosome that it has the whole length of the chromosome for the other twist to fall. Consequently, it is to that region which we will pay most attention in our subsequent analysis. Some other difficulties, such as genetic variations of crossing over, which if present materially influence our conclusions, will be the first to receive consideration.

FREQUENCY DISTRIBUTIONS OF THE VARIATION IN PERCENT OF SINGLE AND DOUBLE CROSSING OVER IN THE THIRD CHROMOSOME OF DROSOPHILA

The frequency of the percentage of crossing over for the various regions is shown in tables 1 and 2, both in absolute figures and in percentages.

TABLE 1

Percentage of single crossing over ($\frac{s_e s_s c'' r_o}{D'}$).

Per- cent crossing over	Region 1		Region 2		Region 3		Region 4	
	Fre- quency	Per- cent	Fre- quency	Per- cent	Fre- quency	Per- cent	Fre- quency	Per- cent
0-2	2	.83	2	.83	1	.41		
2-4	4	1.66	10	4.16	9	3.75		
4-6	18	7.50	30	12.51	18	7.50		
6-8	29	12.08	51	21.26	45	18.76	3	1.25
8-10	44	18.34	57	23.76	57	23.76	2	.83
10-12	55	22.93	31	12.92	44	18.34	5	2.09
12-14	38	15.84	27	11.25	29	12.08	13	5.41
14-16	30	12.51	16	6.66	19	7.91	15	6.25
16-18	13	5.41	9	3.74	11	4.59	38	15.84
18-20	3	1.25	5	2.09	4	1.66	34	14.17
20-22	3	1.25	1	.41	3	1.25	48	20.00
22-24	1	.41	1	.41			28	11.67
24-26							23	9.58
26-28							17	7.08
28-30							7	2.92
30-32							5	2.09
32-34								
34-36							1	.41
36-38							1	.41
Total	240	100.00	240	100.00	240	100.00	240	100.00

In engaging in any discussion of the distributions and the interrelations between them, it seemed advantageous to have the physical constants, mean, standard deviation, and coefficient of variation, before us. In the calculations of these constants SHEPPARD'S correction for the second moment was not used.

A number of interesting points are brought out by this table:

1. It will be seen that the crossing-over ratio for $D's_s$ obtained by summation of the values for $D'c_u$ and $c_u s_s$ that the sum is only 8.388 percent, as against 9.483 percent for the cross which does not contain

TABLE 2
Percentage of double crossing over ($\frac{s_0 \quad s_1 \quad e^2 \quad r_0}{D'}$).

Percent double crossing over	Region 1, 2		Region 1, 3		Region 1, 4		Region 2, 3		Region 2, 4		Region 3, 4	
	Fre- quency	Per- cent	Fre- quency	Per- cent	Fre- quency	Per- cent	Fre- quency	Per- cent	Fre- quency	Per- cent	Fre- quency	Per- cent
0.0-0.5	96	40.00	92	38.31	35	14.59	152	63.34	67	27.91	154	64.17
0.5-1.0	45	18.76	26	10.84	14	5.84	42	17.50	31	12.92	39	16.25
1.0-1.5	27	11.25	38	15.84	40	16.66	26	10.83	41	17.09	31	12.93
1.5-2.0	30	12.51	30	12.51	38	15.84	11	4.59	32	13.33	5	2.09
2.0-2.5	16	6.66	19	7.92	33	13.75	5	2.09	24	10.01	6	2.50
2.5-3.0	16	6.66	18	7.51	20	8.33	1	.41	13	5.41	2	.83
3.0-3.5	3	1.25	5	2.09	20	8.33	1	.41	13	5.41	1	.41
3.5-4.0	3	1.25	5	2.09	14	5.84	2	.83	11	4.59	1	.41
4.0-4.5			1	.41	12	5.00			2	.83		
4.5-5.0	4	1.66	3	1.25	5	2.09			5	2.09		
5.0-5.5			2	.83	4	1.67						
5.5-6.0					1	.41					1	.41
6.0-6.5					2	.83						
6.5-7.0			1	.41					1	.41		
7.0-7.5												
7.5-8.0												
8.0-8.5												
8.5-9.0					2	.83						
Total	240	100.00	240	100.00	240	100.00	240	100.00	240	100.00	240	100.00

TABLE 3
Physical constants for the frequency distributions of the third chromosome.

Region	Mean	Standard deviation	Coefficient of variation
$s_s D'§$	10.900 ± .166	3.807 ± .117	34.923 ± 1.199
$s_s s_n^*$	20.383 ± .276	6.332 ± .201	31.003 ± 1.074
$s_s e^{**}$	30.458 ± .361	8.282 ± .263	27.189 ± .917
$s_s r_o^*$	50.858 ± .420	9.654 ± .306	18.623 ± .624
$D' c_u†$	2.845 ± .297	2.680 ± .210	58.956 ± 5.885
$D' s_n§$	9.483 ± .170	3.907 ± .120	41.201 ± 1.468
$D' e^{**}$	19.558 ± .258	5.916 ± .188	30.335 ± 1.034
$D' r_o^*$	39.958 ± .330	7.568 ± .240	18.513 ± .624
$c_u s_n†$	5.543 ± .186	1.677 ± .131	40.823 ± 1.636
$s_s H'†$	9.862 ± .251	2.263 ± .177	23.558 ± .875
$s_s e^{*§}$	10.075 ± .165	3.785 ± .117	37.570 ± 1.310
$s_s r_o^*$	30.475 ± .266	6.103 ± .192	19.427 ± .651
$H' c^{*†}$	1.173 ± .258	2.323 ± .182	62.980 ± 6.414
$e^{**} r_o§$	20.400 ± .216	4.957 ± .153	24.300 ± .791
$s_s D'$ and $D' s_s§$	1.065 ± .045	1.029 ± .032	96.651 ± 5.040
$s_s D'$ and $s_s e^{*§}$	1.273 ± .041	.950 ± .029	74.636 ± 3.341
$s_s D'$ and $e^{**} r_o§$	2.170 ± .063	1.458 ± .045	67.178 ± 2.853
$D' s_n$ and $s_n e^{*§}$.609 ± .027	.613 ± .019	100.766 ± 5.401
$D' s_n$ and $e^{**} r_o§$	1.498 ± .053	1.210 ± .037	80.792 ± 3.777
$s_s e^{**}$ and $e^{**} r_o§$.613 ± .029	.672 ± .021	109.750 ± 6.240

* Compound constants calculated from the separate components by summation.

† Calculated from ungrouped frequencies of table A (Appendix, p. 241).

§ Calculated from grouped frequencies of table D (Appendix, pp. 243-247).

the gene for c_u . Likewise, in the summation of the values $s_s H'$ and $H' e_s$, there is quite a considerable difference from the result obtained in the cross without H' (11.035 to 10.075). It remains for a further section of this paper to discuss whether or not these differences are significant.

2. It will be noted that there is a very high coefficient of variability in practically every ratio, this variability being greatest when the mean crossing over ratio is small. Thus, it may be said that when dealing with factors which separate only rarely there is to be expected great fluctuation in the value of the ratio of crossovers to the total number of flies

3. It is further to be noted that the variability in the number of double breaks is markedly higher than when the variability of the single break is considered. This high variability is no doubt due in part to the small absolute number of double crossovers which are expected. However, this does not in any measure account for the whole of it. From the table

we can safely say that double crossing over is an extremely variable character.

4. A comparative view of this variability will give us a better basis for judging of its real magnitude. A constant is so named because it has a low variability, and as this variability becomes greater, its action is measured by so-called laws. Now, it will be interesting to compare some of the morphological characters which more nearly approach physical characters and some more nearly physiological characters with the values for crossing over.

TABLE 4
Variation constants for various characters.

Subject	Character	Coefficient of variation	Authority
Poland-China swine	Size of litter	27.41	SURFACE (1909)
Man	Number of children	48.14	POWYS (1905)
English males	Heart weight	22.22	GREENWOOD and BROWN (1913)
Cattle	Rev. maximum daily milk yield	18.00	GAVIN (1913)
Domestic fowl	Shell weight of eggs	13.86	CURTIS (1914)
English	Length of skull	3.31	MACDONELL (1904)
Domestic fowl	Breadth of egg	3.29	PEARL (1914)
Drosophila	Single crossing over	18.51-58.96	This paper
Drosophila	Double crossing over	67.18-109.76	This paper

A glance at this table suffices to show how much crossing over in different females varies. Even the lowest values stand well up among the characters which are more purely physiological in character and the highest value is much above that which is ordinarily found even in the physiological characters. Such a high variability demands explanation, and it will be the function of a succeeding section of the paper, where the data has been collected for it, to attempt such explanation.

VARIATION IN CROSSING OVER BETWEEN TWO FIXED POINTS IN EXPERIMENTS CONTAINING OTHER INTERMEDIATE GENES

In undertaking a discussion of crossing over, a matter of prime importance is the question whether or not a change in the genes between two fixed points influences the amount of crossing over between these two fixed points.

It has already been shown by STURTEVANT (1917) and MULLER (1916) that there are disturbing factors in the second and third chromosomes which reduce the crossing over of the factors located in their re-

spective chromosomes. In every case these disturbing factors have quite a considerable effect, as, for example, the cutting down of crossing over from about 50 percent to 1 percent. Now the question arises, do all factors influence crossing over? Is an effect on crossing over as much a function of a gene as the eye color or the body color that is given to an animal by its presence? A partial answer to this problem may be had by a comparison of the crossing-over value for two sets of data in which it is known that in the first set a given gene is present which is not present in the second set. To this end the following data were collected in which heterozygous females of the composition indicated for each distribution were crossed with males homozygous for the recessives carried by the female.

It will be seen on examination of these tables that they differ from each other in having a different set of factors run in combination with certain common factors. Thus, table 5 differs from table 6 in being formed from a cross which has a chromosome carrying *dichaete* sub-

TABLE 5

$$\text{Genes, } \frac{s_e \quad s_s \quad c^a \quad r_o}{D'}$$

Non-crossovers	Single crossovers				Double crossovers					
0	1	2	3	4	1, 2	1, 3	1, 4	2, 3	2, 4	3, 4
17,171	2,208	2,211	2,639	5,163	292	392	629	132	413	125

Triple crossovers				Quadruple crossovers	Total
1, 2, 3	1, 2, 4	1, 3, 4	2, 3, 4	1, 2, 3, 4	
11	39	17	13	1	31,456

TABLE 6

$$\text{Genes, } \frac{s_e \quad s_s \quad c^a \quad r_o}{H'}$$

Non-crossovers	Single crossovers				Double crossovers						Triple crossovers		Total
0	1	2	3	4	1,2	1,3	1,4	2,3	2,4	3,4	1,2,3	1,2,4	
1459	378	175	29	436	31	8	89	10	10	2	3	1	2631

TABLE 7
 $D' \quad p^p \quad s_s \quad e^e \quad r_o$
Genes,

Non-crossovers	Single crossovers				Double crossovers						Triple crossovers			Total
0	1	2	3	4	1,2	1,3	1,4	2,3	2,4	3,4	1,2,3	1,2,4	1,3,4	
1220	92	263	312	366	15	25	30	9	32	11	1	4	1	2381

TABLE 8
 $s_c \quad c_u \quad s_s \quad e^e \quad r_o$
Genes,
 $D' \quad \quad \quad H'$

Non-crossovers	Single crossovers						Double crossovers								
0	1	2	3	4	5	6	1,2	1,3	1,4	1,5	1,6	2,3	2,4	2,5	2,6
4664	534	112	319	689	77	1320	22	25	55	14	150	16	22	2	46

Double crossovers				Triple crossovers									Total
3,4	3,5	3,6	4,6	1,2,3	1,2,5	1,2,6	1,3,4	1,3,6	1,4,6	2,3,6	3,4,6	3,5,6	
3	1	44	37	1	1	1	1	3	1	5	1	1	8167

stituted for one carrying hairless. Table 5 differs from table 8 in having a chromosome carrying dichaete substituted for one carrying both dichaete and hairless.

This, then, gives the data necessary to test out the previous question, Is an effect on crossing over as much a part of the function of a gene as the character produced by it? For if the gene is not an integral part of the mechanism, but is simply carried along by it, it would be expected that a gene's presence would have no effect on crossing over. But if allelomorphs are granules of varying physical characteristics in the chromosome and crossing over takes place by the breaking of finely spun-out twisted threads, it would be expected that one type of granule substituted for another type of granule would affect the position and number of breaks in a chromosome, much the same as the breaking of the strands of a wire cable in a given place is influenced by whether German silver or steel occupies that place. All that is necessary is to compare the constant elements of these distributions as they would have been, supposing

that the gene in question had not been there. To compare the cross *dichaete* heterozygous with *hairless* heterozygous all that is necessary is to reduce the distribution of table 5 to what it would have been in a cross $s_e s_e k e^+ r_o$ neglecting *dichaete* and *hairless* as in table 9. As it was impossible to follow the kidney factor (*k*) in the presence of rough, it was not counted.

TABLE 9

Formula of back-crossed female	Reduced formula	0	1	2	3	1, 2	1, 3	2, 3	1,2,3	Total
A. $\frac{s_e s_e e^+ r_o}{D'}$	$\frac{s_e s_e e^+ r_o}{D'}$	17463	4419	2650	5202	524	1042	126	30	31456
B. $\frac{s_e s_e e^+ r_o}{H'}$	$\frac{s_e s_e e^+ r_o}{H'}$	1469	381	197	443	39	89	12	1	2631

TABLE 10

Reduction of crossing over of table 9 to single crossover in each region.

Formula of back-crossed female	Reduced formula	0	1	2	3
A. $\frac{s_e s_e e^+ r_o}{D'}$	$\frac{s_e s_e e^+ r_o}{D'}$	17463	6015	3330	4600
B. $\frac{s_e s_e e^+ r_o}{H'}$	$\frac{s_e s_e e^+ r_o}{H'}$	1469	420	249	543

Without going into further detail, the eight possible arrangements of these five tables were made for the comparison of the effect of their different factors on the crossover ratios between the common factors. The measure used for this comparison was the well-known χ^2 test of PEARSON. Since none of these were theoretically fitted frequency curves, the comparison was made only between the non-crossovers and single crossovers (obtained by summation) of each distribution. In this way the danger of *q* being small in the case of double crossovers is avoided. In the following table are given the results of the comparisons of these distributions.

From this it is seen that even taking the greatest probability (that of the two curves *D'* heterozygous and *H'* heterozygous being the same), the odds against any of these curves coming from the same population are all more than three times the probable error (25 to 1). The disturbance

TABLE 11

Distributions compared	χ^2	P
$\frac{s_e \ s_n \ k \ c^* \ r_o}{D'}$ and $\frac{s_e \ s_n \ k \ c^* \ r_o}{H'}$	8.37	Less than .039859
$\frac{s_e \ s_n \ k \ c^* \ r_o}{D'}$ and $\frac{D' \ p^b \ s_n \ k \ c^* \ r_o}{D'}$	234.76	$1/10^{40}$
$\frac{s_e \ s_n \ k \ c^* \ r_o}{D'}$ and $\frac{s_e \ c_u \ s_n \ k \ c^* \ r_o}{D' \ H'}$	82.81	$1/10^{14}$
$\frac{s_e \ c_u \ s_n \ k \ c^* \ r_o}{D' \ H'}$ and $\frac{s_e \ s_n \ k \ c^* \ r_o}{H'}$	29.94	.000005
$\frac{s_e \ c_u \ s_n \ k \ c^* \ r_o}{D' \ H'}$ and $\frac{D' \ p^b \ s_n \ k \ c^* \ r_o}{D'}$	307.05	$1/10^{64}$

does not necessarily confine itself to the region occupied by the gene, as a study of the distributions will show. It may be anywhere and its effect may be more or less pronounced. In general, however, the effect is not as great as that of the previously found modifiers for crossing over. From this it follows that each gene has been accompanied by a disturbance in the crossing-over mechanism. This disturbance, it seems to me, may be due to the difference in the strains and stresses set up in the chromosome by different types of particles.

The essential conclusions to be drawn from this section of the work is that only those experiments containing as nearly as possible the same genes can be used in any critical study of crossing over and that the amount of crossing over between two fixed genes is a variable quantity, depending on the genes which are present. This does not mean that the crossing-over ratio is not a good means of measuring the position of the factors in a chromosome, it merely means that the scale from experiment to experiment may vary. Thus we should carefully consider the factors present in every experiment.

DOES FOOD OR SEASON INFLUENCE CROSSING OVER?

To test this, it becomes necessary to divide our records (table D) at the places where a change of food occurred. The distributions resulting may then be compared by means of the previously described test, χ^2 , for similarity. Table 12 gives the distributions and the χ^2 with the resulting probabilities that the various distributions can come from random sampling. Thus the χ^2 value of 9.78 obtained by the comparison of the distribution resulting from the use of fermented banana and of a starch-

sugar mixture for food indicates that once in 22 such trials as good or worse a fit would be expected.

TABLE 12

		0	1	2	3	4	Total	Distribu- tions compared	χ^2	P
Fermented banana	I	4456	866	744	779	1691	8536	I & II	9.78	.045
Starch-sugar	II	4944	1009	922	959	1824	9658	II & III	5.78	.219
mixture	III	5797	1193	962	1132	2159	11244	I & III	6.91	.147

This gives a fair chance that all three curves are samples from the same population as would be expected *a priori*. The second and third distributions are the much better-fitting divisions. From this it may be concluded that the food used has little or no effect on crossing-over. Since the divisions may also represent divisions for different seasons, it follows from this that seasons have little or no effect on crossing over unless one takes the doubtful stand that the effect of food and season exactly counterbalance each other.

CROSSING OVER IN RELATION TO MODIFYING FACTORS

It is too obvious to require experimental demonstration that modifying factors for crossing over would influence in a marked way the data obtained for crossing over in a large number of females where such modifying factors were present.

In an earlier section of this paper it has been shown that such a crossing-over disturbance does occur when known and accompanying unknown genes are introduced between fixed points. Now, it is conceivable that there are unknown genes in any cross which may cause crossing-over disturbances and will be distributed unevenly in the female whose offspring are counted. This uneven distribution will make the results from such a cross heterogeneous. The test of whether or not the material to be used in the succeeding study of crossing over is homogeneous will be dealt with in this section.

If we select only the females giving the lowest total percent of crossing over in the chromosome in question, we should lower the percent of crossing over if modifying factors are present. Such a selection experiment has been performed, the selection continuing for six generations of strictly brother-and-sister matings. In some respects these data are unsatisfactory. The chief difficulty lies in the few individuals that it was

possible to include in a given generation. In this way a large individual variation is possible which may in some cases obscure the results. However, taken as a whole, I think it will be found to answer the question, for the constants are rather uniformly the same in showing no effect of selection.

The material chosen for this selection experiment was the cross, male homozygous for sepia, spineless, kidney, sooty, rough, mated to a female heterozygous dichæte and sepia, spineless, kidney, sooty, rough. This cross is chosen as it is to be the cross used in the study of the mechanism of crossing over which is to follow. The reductions of the data to their means and correlations for each given generation are tabulated in table 13.

TABLE 13

Generation	1	2	3	4	5
Mean total of crossing over	37.97±.970	47.81±1.274	49.67±1.929	49.03±1.272	55.75±1.09
	1 and 2	2 and 3	3 and 4	4 and 5	
Parent-and-offspring correlations	.1033±.1423	.1595±.1756	.3360±.1348	-.2107±.1177	

A like experiment has been made for selection of females with high percent of crossing over; the results are seen in table 14.

TABLE 14

Generation	1	2	3	4	5	6
Mean	58.07±1.150	51.52±1.717	52.57±.587	49.85±2.769	51.16±.929	54.80±2.
	1 and 2	2 and 3	3 and 4	4 and 5	5 and 6	
Parent-and-offspring correlations	-.0641±.1105	.0297±.3369	.1500±.2484	-.1348±.1912	-.0780±.2021	

It is evident from this data that while the probable errors are large, the data are in accord in showing no effect of selection. This is even more striking when one considers the individual pedigrees where one generation may jump to the other extreme from its parent.

In this connection, it seems to me especially instructive to compare the correlations obtained by MACDOWELL (1917) during his selection experiment with those we have here. After selection had been continued for

more than fifty generations, the parent and offspring correlations in one set of experiments for the bristle number were -0.1436 for the males and -0.1378 for the females, or correlation of about the same magnitude as those in the experiment given above. Thus we see that in actual magnitude the correlations are about the same size for our experiment as those

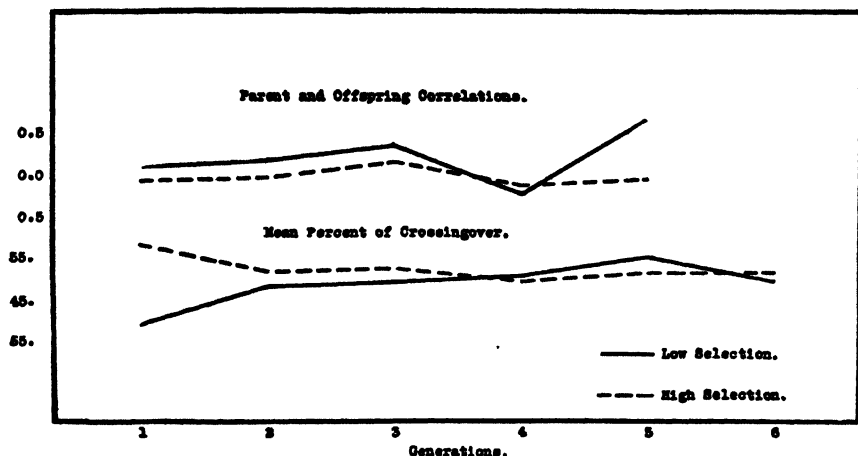


DIAGRAM 2

for an experiment carried on for some fifty-four generations where it was shown that selection had no appreciable effect after the first six generations. It seems a justifiable conclusion to be drawn from the above, the origin of the stock from a single pair, and the subsequent long inbreeding, that the records are homogeneous and without heterozygous modifying factors for either reduction or increase in crossing over. The crossing-over mechanism is, then, working in a system of events controlled only by the mechanism used in crossing over for the particular set of factors.

ON THE RELATION BETWEEN NUMBER OF OFFSPRING AND VIABILITY OF THE FACTOR COMBINATIONS USED

As an explanation of the difference of expected from obtained ratios in some crosses, the hypothesis of differential viability has been proposed. This is on the face of it a legitimate hypothesis for such results, as it is known that some combinations of factors are less viable than others. Now, in a bottle of a large number of flies, where crowding takes place, it is an easy step to consider that in the competition more of the less viable combinations of factors die. Since this cause might be a disturbing

influence in the ratios obtained in these experiments, if one combination of factors were less viable than another, it becomes necessary to test for disturbances of linkage due to inviability. If it is considered that the number of flies produced by a bottle is a suitable measure of its condition, then if there is a disturbance of the linkage due to the viability in any given combination with variations in food or crowding, it would be expected that the ratios obtained for such bottles would be correlated. If we find no such correlation it is justified to conclude that no such difference in viability of factors remained unbalanced in our experiments. Table 15 shows the correlation for the given combinations of factors and the bottle output.

TABLE 15

Region	Correlation	Region	Correlation
1	.0670 \pm .0433	1, 3	.1115 \pm .0429
2	-.0622 \pm .0433	1, 4	.0299 \pm .0434
3	.1174 \pm .0429	2, 3	-.0410 \pm .0434
4	.0275 \pm .0434	2, 4	-.0658 \pm .0433
1, 2	.0119 \pm .0434	3, 4	-.0436 \pm .0434

Thus it will be seen that only in two cases does the correlation run as high as 0.1. Even this is only slightly above two times the probable error and therefore cannot be considered as significant. We may conclude with safety, then, that in no combination of factors which resulted from the crosses that were used did unbalanced differential viability exist.

The general conclusions which may be drawn from the first part of this paper are:

1. Crossing over between two fixed points is highly variable both relatively and absolutely.

2. A change in genes between two or more fixed points in the third chromosome may be accompanied by a slight disturbance of the crossing-over ratios between these fixed points.

3. The food used had no effect on crossing over.

4. It is highly probable that there were no unequally distributed modifying factors for crossing over at work in our data for the back-crossed females heterozygous for *dichaete* and *sepia*, *spineless*, *kidney*, *sooty*, *rough*, to be used for the critical study of crossing over in the succeeding section.

5. There is no effect of unbalanced differential viability resulting from any of our combinations of factors.

ON THE RELATION OF CROSSING OVER TO POSITION

In any study of the crossing-over mechanism an adequate analysis of the problem must include a knowledge of the relation between the total crossing over in one part of the chromosome and the total crossing over in the remaining regions. Toward the solution of this phase of the problem the data in table D (page 243) have been collected. The cross of a homozygous sepia spineless kidney sooty rough male mated to a heterozygous female sepia spineless kidney sooty rough on one side, dichæte on the other, is the same as that used in our selection experiment where the stock was shown to be free from factors modifying the crossing-over ratios. For want of space the formed correlation tables have been omitted. All correlation coefficients have been calculated by the usual formula,

$$r = \frac{s(xy)}{N\sigma_x\sigma_y}$$

The problem presents some difficulties as the above formula does not give the true correlation as uninfluenced by other associated variables. For obtaining these it is necessary to resort to the use of partial correlations. The fundamental correlations are shown in table 16 with their probable errors calculated by the help of the tables edited by PEARSON (1914).

TABLE 16

Coefficients of correlation for total crossing over in the different regions of the chromosome, together with those for number of offspring.

Section	Coefficient of correlation	Section	Coefficient of correlation
1 and 2	0.7136 ± .0213	2 and 4	0.5985 ± .0280
1 and 3	0.7888 ± .0164	2 and T*	0.6628 ± .0244
1 and 4	0.7426 ± .0195	3 and 4	0.7410 ± .0196
1 and T*	0.8108 ± .0149	3 and T	0.8335 ± .0133
2 and 3	0.6761 ± .0236	4 and T	0.8782 ± .0100

* T stands for the total offspring output per female.

In every case the correlations are high and many times their probable errors. This table furnished us the material for calculation of the correlations between the different regions when the effect of difference in number of offspring per mating, on the correlation between two given chromosome regions, is eliminated through the use of partial correlations.

TABLE 17
Partial correlations deduced from table 16.

Regions correlated	Partial correlations (Total constant)
1 and 2 .T	.4019 \pm .0365
1 and 3 .T	.3492 \pm .0382
1 and 4 .T	.1093 \pm .0430
2 and 3 .T	.2988 \pm .0396
2 and 4 .T	.0458 \pm .0435
3 and 4 .T	.0341 \pm .0435

The correlations range from 0.4019 to 0.0341, from nine times the probable error to insignificance. An interesting relationship is apparent in this range of correlation. Considering any given region correlated with the remaining regions, the algebraic value of the correlation coefficients is greatest for the first left-hand region and diminishes toward the right hand. This is clearly brought out in table 18, together with the differences and their probable errors calculated by the usual difference formulae.

TABLE 18
Correlation and differences for the single crossovers of successive chromosome segments.

Regions	Coefficient of correlation	Difference	Regions	Coefficient of correlation	Difference
1 and 2 .T	.4019		3 and 1 .T	.3492	
1 and 3 .T	.3492	.0527 \pm .0528	3 and 2 .T	.2988	.0504 \pm .0550
1 and 4 .T	.1093	.2399 \pm .0575	3 and 4 .T	.0341	.2647 \pm .0584
2 and 1 .T	.4019		4 and 1 .T	.1093	
2 and 3 .T	.2988	.1031 \pm .0539	4 and 2 .T	.0458	.0635 \pm .0611
2 and 4 .T	.0458	.2530 \pm .5876	4 and 3 .T	.0341	.0117 \pm .0615

The significance of these differences becomes apparent by a comparison with their probable errors. In each case the relation of the crossing over between the 1 and 3, 2 and 3, and 3 and 2, with the fourth 1 and 4, 2 and 4, and 3 and 4, is quite significant. The other values do not have such great significance, some of the greater difference in the case of the fourth region may be due to its greater length. The consistency of the relationship leads one to suspect that it is more than chance, even though the differences are not great in comparison with the probable errors.

The fact that all of the eight differences between adjoining sections arranged in order from left to right are positive when the likelihood *a priori* is equal, as to whether any given difference shall be an excess or defect, is greatly in favor of the view that this is not a chance relationship, but is one brought about by some inherent cause in the mechanism of crossing over. Taken at its face value, this graded scale of correlation means that the conditions in a given female favorable to a given grade of crossing over in the first region is in a less degree favorable to crossing over in the second region and to a still less degree to crossing over in the regions to the right of the second.

It is further brought out that the distribution of the single crossovers for the different segments of the chromosome is by no means a random sample. They are associated variates. This conclusion is important in a number of ways. The chief among these is that in treating any expectation for double crossovers as the product of the single crossovers, either as just the single observed crossovers or as the single crossovers obtained by summation, we are committing a grave error. The error lies in the fact that we do not take into consideration that they are in general correlated variates.

Because of the importance of this correlation and to further test its generality another experiment was performed, using a larger number of factors. The cross used for this was a male homozygous for sepia, curled, spineless, kidney, sooty, rough, mated to a female heterozygous for dichaete, hairless on one side, and sepia, spineless, kidney, sooty, rough, on the other. It is realized that this distribution is not comparable with the preceding one, yet should the mechanism of crossing over be the same, the manner of crossing over in the two cases should be alike, even though the absolute values were different.

The correlations for this cross are given below. It is realized only too keenly that they are based on rather small numbers; as measured by the standard of the probable error, however, they are significant. Not only that, but taken in consideration with the preceding data it is believed as greater numbers are gathered that the correlations will remain practically the same except for a little tendency to smooth. Table 19 gives the correlation for the successive regions calculated from the ungrouped frequencies by the use of the ordinary formula.

By examination of this table it is seen that it substantiates a former conclusion that the crossing-over values for various sections of the chromosome may be, and generally are, correlated variates. This corre-

TABLE 19
Coefficients of correlation for the successive regions of the third chromosome.

Section	Coefficient of correlation	Section	Coefficient of correlation
<i>s</i> _o <i>D'</i> and <i>D'</i> <i>c</i> _u * <i>s</i> _o	0.5792 ± .0677	<i>D'</i> <i>c</i> _u and <i>e</i> _s <i>r</i> _o	0.0640 ± .1004
<i>s</i> _o <i>D'</i> and <i>c</i> _u <i>s</i> _o	0.3100 ± .0911	<i>c</i> _u <i>s</i> _o and <i>s</i> _o <i>H'</i>	0.1945 ± .0969
<i>s</i> _o <i>D'</i> and <i>s</i> _o <i>H'</i>	0.4440 ± .0809	<i>c</i> _u <i>s</i> _o and <i>H'</i> <i>e</i> ^a	0.2752 ± .0932
<i>s</i> _o <i>D'</i> and <i>H'</i> <i>e</i> ^a	-0.0070 ± .1003	<i>c</i> _u <i>s</i> _o and <i>e</i> _s <i>r</i> _o	0.2023 ± .0967
<i>s</i> _o <i>D'</i> and <i>e</i> _s <i>r</i> _o	0.1920 ± .0971	<i>s</i> _o <i>H'</i> and <i>H'</i> <i>e</i> ^a	-0.2003 ± .0967
<i>D'</i> <i>c</i> _u and <i>c</i> _u <i>s</i> _o	0.2224 ± .0958	<i>s</i> _o <i>H'</i> and <i>e</i> ^a <i>r</i> _o	0.1194 ± .0994
<i>D'</i> <i>c</i> _u and <i>s</i> _o <i>H'</i>	0.3970 ± .0849	<i>H'</i> <i>e</i> ^a and <i>e</i> ^a <i>r</i> _o	-0.2364 ± .0952
<i>D'</i> <i>c</i> _u and <i>H'</i> <i>e</i> ^a	-0.1330 ± .0990		

* The total number of offspring for each section held constant by the method of partial correlations.

lation runs as high as 0.5792 and drops to -0.2364. The correlation for the *H'e*^a distance is seen in most cases to be abnormal. This is probably due to the fact that the ratio for crossing over of this section is rather small in absolute magnitude and since there are only a few crossovers expected with the number of individuals small, the crossing-over values are subject to considerable variation. For this reason where more individuals are included in the data this discrepancy will straighten out and fall in line with the observations previously made. This conclusion is justified, it is thought, for when the data are plotted the curve of the successive regional correlations of the smaller series onto the curve of the successive regional correlations for the larger series of data, the curve of the larger series is seen to bisect the fluctuations of the smaller series as would be expected if they were samples of like populations governed by the same mechanical laws. Thus, barring the slight modifications of the double-crossover values caused by the kind of intermediate factors present, in general the relationship previously established is seen to hold.

Thus, there is a correlation between the crossovers in a given region and the crossovers in successive regions. In general, this relation between crossing over in the various regions is greatest between the region toward the left-hand end of the chromosome and the region under consideration. That is, high crossing over in region BC is more likely to be correlated with high crossing over in AB than in the region of CD.

ON THE RELATION BETWEEN CROSSING OVER IN ONE REGION AND
CROSSING OVER INCLUDING TWO REGIONS AT A TIME

The question having most immediate bearing on the mechanism of crossing over is: What is the relation between crossing over in one region and crossing over including two regions at a time, for the successive segments of the chromosome? The importance of the question lies in the fact that if it can be shown that the value of the double crossovers falls in the ordinary cocked-hat frequency curve, it indicates that there is a definite modal length for maximum amounts of double crossing over. Such a modal length is accounted for by the twisting hypothesis of crossing-over as due to the chromosome threads lying across each other in loose twists during the stage at which crossing over takes place. If it can be shown that the double crossing-over ratios do rise and fall for each of the successive segments of the chromosome studied, it not only strengthens the twisting hypothesis, but puts the only other existing hypothesis of reduplication in the forced position of adopting another co-hypothesis to account for the fact, as has been shown in the previous discussion of the theory. To make a study of the crossing-over relationship secure and to be sure that our deductions are not based on a false groundwork, it is necessary to carefully consider how the experiment is performed. All factors that are to be compared should be put together in the same experiment and only data known to be alike in modifying factors used. Such data are at hand in the data on back-crossed females contained in our records for the back-crossed female sepia, spineless, kidney, sooty, rough, on one side, the dominant dichæte on the other, as has been shown by the previous parts of this paper. Before beginning the mathematical analysis of the data it may be well to consider some general aspects of the material as presented. There is one difficulty which must be taken into consideration when considering all measurements containing the region e^*r_o , that is, this region is about twice the value of the other regions under consideration. Unfortunately, it was impossible to get this region broken up into smaller parts, for as yet no good factor is known to occupy this region. This high ratio of crossing over means that if the length of the double crossing over is of a value such that the two extremes of the long e^*r_o region fall so as to include two segments, the data will have a bimodal distribution and our observed correlation will have a distorted value. This possible distortion should be kept in mind when considering the data, and in general the main conclusions should rest on observations of the shorter distances.

The first difficulty confronted is the fact that the single crossovers in the various regions of the chromosome are correlated variates partly dominated in their position of breaking by some such cause as the plasma surrounding them. Thus it becomes necessary in a critical experiment to determine the mode of distribution of the breaks in a chromosome, to keep the single-crossover correlations constant throughout the length of the chromosome in females used in the experiment (constant with regard to its quality to influence double crossing over). Since the influence of this relation has been measured by the correlations of the different regions of the chromosome with one another, a mathematical universe may be formed to measure the double crossovers as they would have occurred in an experiment where the surroundings of the chromosomes are constant. In other words, in this way the obstacle of differential single crossing over as regards the various regions of the chromosome is removed. When this is done it is possible to measure the relation that exists in the double crossovers of the successive regions of the chromosome, knowing that it is only the effect of the chromosome mechanism that is studied.

To the end of establishing such a universe, it is necessary to obtain the relationship between the successive regions of the chromosome for double crossing over as they occur in a universe affected by the correlation of the single crossovers for the different regions. To this end the data given in table 20 were collected.

TABLE 20
Correlations between single and double crossovers and number of offspring per mating.

Doubles	Region 1 correlation	Region 2 correlation	Region 3 correlation	Region 4 correlation	Total offspring
1, 2	0.5578 \pm .0300	0.6336 \pm .0260	0.4421 \pm .0350	0.3634 \pm .0378	0.3857 \pm .0371
1, 3	0.7724 \pm .0176	0.6239 \pm .0266	0.7073 \pm .0218	0.5640 \pm .0297	0.6180 \pm .0269
1, 4	0.6483 \pm .0253	0.4804 \pm .0335	0.5730 \pm .0292	0.6202 \pm .0268	0.6224 \pm .0267
2, 3	0.1795 \pm .0421	0.3572 \pm .0380	0.3285 \pm .0388	0.1375 \pm .0427	0.1734 \pm .0422
2, 4	0.4810 \pm .0335	0.6258 \pm .0265	0.4472 \pm .0348	0.4907 \pm .0330	0.4249 \pm .0357
3, 4	0.4220 \pm .0358	0.4269 \pm .0356	0.5336 \pm .0311	0.4489 \pm .0348	0.3830 \pm .0372

Before tabulating the constants which will be necessary to the final study, the establishing of the linearity of regression for the tables from which the fundamental correlations of table 16 and table 20 have been deduced, is necessary. The constants to determine this are given in table 21.

TABLE 21
Criteria for linearity of regression.

Characters correlated	r	η	$\eta - r$	Σ_m	$\eta^2 - r^2$
T and 1	.8108 \pm .1149	.8275 \pm .0137	.0167	.3287 σ	.0274 \pm .0143
T and 2	.6628 \pm .1244	.6733 \pm .0238	.0109	.2353 σ	.0140 \pm .0102
T and 3	.8335 \pm .1133	.8439 \pm .0125	.0104	.2611 σ	.0173 \pm .0114
T and 4	.8782 \pm .0700	.8847 \pm .0094	.0066	.2127 σ	.0115 \pm .0093
T and 1, 2	.3857 \pm .0171	.4085 \pm .0363	.0228	.2674 σ	.0181 \pm .0115
T and 1, 3	.6180 \pm .0269	.6563 \pm .0248	.0383	.4019 σ	.0488 \pm .0187
T and 1, 4	.6224 \pm .0267	.6394 \pm .0258	.0172	.2993 σ	.0227 \pm .0123
T and 2, 3	.1734 \pm .0422	.1971 \pm .0418	.0237	.1862 σ	.0088 \pm .0081
T and 2, 4	.4249 \pm .0357	.4436 \pm .0350	.0187	.2532 σ	.0162 \pm .0109
T and 3, 4	.3830 \pm .0372	.4008 \pm .0365	.0178	.2347 σ	.0140 \pm .0102
1 and 2	.7136 \pm .0213	.7307 \pm .0203	.0172	.2722 σ	.0247 \pm .0135
1 and 3	.7888 \pm .0164	.7993 \pm .0157	.0105	.2241 σ	.0167 \pm .0112
1 and 4	.7426 \pm .0165	.7472 \pm .0192	.0046	.1437 σ	.0069 \pm .0072
1 and 1, 2	.5578 \pm .0300	.5752 \pm .0291	.0174	.2438 σ	.0197 \pm .0121
1 and 1, 3	.7724 \pm .0176	.7788 \pm .0171	.0064	.1730 σ	.0099 \pm .0086
1 and 1, 4	.6483 \pm .0253	.6728 \pm .0239	.0245	.3123 σ	.0324 \pm .0154
1 and 2, 3	.1795 \pm .0421	.2227 \pm .0414	.0431	.2288 σ	.0174 \pm .0113
1 and 2, 4	.4810 \pm .0331	.4920 \pm .0330	.0110	.1797 σ	.0107 \pm .0089
1 and 3, 4	.4220 \pm .0351	.4796 \pm .0335	.0576	.3957 σ	.0519 \pm .0190
2 and 3	.6761 \pm .0236	.6847 \pm .0231	.0086	.1662 σ	.0117 \pm .0094
2 and 4	.5985 \pm .0286	.6238 \pm .0266	.0253	.2702 σ	.0309 \pm .0150
2 and 1, 2	.6336 \pm .0266	.6647 \pm .0243	.0211	.3086 σ	.0404 \pm .0171
2 and 1, 3	.6293 \pm .0266	.6580 \pm .0247	.0341	.3213 σ	.0437 \pm .0177
2 and 1, 4	.4804 \pm .0335	.5289 \pm .0314	.0485	.3399 σ	.0490 \pm .0185
2 and 2, 3	.3572 \pm .0380	.3724 \pm .0375	.0152	.1618 σ	.0111 \pm .0091
2 and 2, 4	.6258 \pm .0265	.6358 \pm .0259	.0100	.1725 σ	.0126 \pm .0097
2 and 3, 4	.4269 \pm .0356	.5018 \pm .0326	.0749	.4052 σ	.0690 \pm .0217
3 and 4	.7410 \pm .0196	.7483 \pm .0192	.0073	.1782 σ	.0109 \pm .0089
3 and 1, 2	.4421 \pm .0350	.4608 \pm .0343	.0187	.2220 σ	.0169 \pm .0112
3 and 1, 3	.7073 \pm .0218	.7161 \pm .0212	.0089	.1912 σ	.0125 \pm .0097
3 and 1, 4	.5730 \pm .0292	.6000 \pm .0279	.0270	.3040 σ	.0317 \pm .0152
3 and 2, 3	.3285 \pm .0388	.3618 \pm .0378	.0333	.2493 σ	.0230 \pm .0124
3 and 2, 4	.4472 \pm .0348	.4632 \pm .0342	.0160	.2063 σ	.0146 \pm .0104
3 and 3, 4	.5336 \pm .0311	.5584 \pm .0291	.0247	.2813 σ	.0271 \pm .0139
4 and 1, 2	.3634 \pm .0378	.3966 \pm .0367	.0332	.2229 σ	.0252 \pm .0135
4 and 1, 3	.5640 \pm .0297	.5857 \pm .0286	.0217	.2873 σ	.0249 \pm .0135
4 and 1, 4	.6202 \pm .0268	.6459 \pm .0249	.0257	.2533 σ	.0325 \pm .0157
4 and 2, 3	.1374 \pm .0427	.1846 \pm .0420	.0471	.1730 σ	.0152 \pm .0106
4 and 2, 4	.4907 \pm .0330	.5133 \pm .0321	.0226	.2114 σ	.0227 \pm .0129
4 and 3, 4	.4489 \pm .0348	.4837 \pm .0333	.0348	.2530 σ	.0325 \pm .0153

It will be remembered that a regression to be linear must have the constants $\eta - r$, $\eta^2 - r^2$ and Σ_m equal to zero within the limits of random

sampling. To obtain these, two new constants must be derived. The correlation ratio is obtained by the formula

$$\eta = \frac{\sigma_{my}}{\sigma_y}$$

where σ_{my} is the standard deviation of the weighted means of the y arrays about the mean of the population. Σ_m , the square root of the mean square deviation of the means of the arrays from the regression line, is derived by the formula

$$\Sigma_m = \sigma_y \sqrt{\eta^2 - r^2}$$

due to PEARSON (1905). The probable errors of $\eta^2 - r^2$ are calculated by the method of BLAKEMAN (1905).

The net result of the study of table 21 shows that all the tables on which our correlations are based have the regression lines linear. In no case is the value of the constant $\eta^2 - r$ greater than three times its probable error.

The point of linearity of regression established, we may now return to table 20. From these constants the singular partial correlation coefficients where the total offspring output per female is held constant, have been tabulated in table 22.

TABLE 22

Partial correlation coefficients for total single and total double crossing over in the third chromosome.

Doubles	Singles: Region 1 correlation Partial correlations	Region 2 correlation Partial correlations	Region 3 cor- relation, Par- tial correlation	Region 4 cor- relation, Par- tial correlation
1, 2 .T	0.4539 \pm .0346	0.5471 \pm .0305	0.2365 \pm .0411	0.0560 \pm .0434
1, 3 .T	0.5895 \pm .0284	0.3640 \pm .0378	0.4423 \pm .0350	0.0564 \pm .0434
1, 4 .T	0.3136 \pm .0393	0.1158 \pm .0430	0.1271 \pm .0429	0.1966 \pm .0419
2, 3 .T	0.0676 \pm .0433	0.3286 \pm .0388	0.3352 \pm .0386	—0.0313 \pm .0435
2, 4 .T	0.2576 \pm .0406	0.5077 \pm .0323	0.1860 \pm .0420	0.2714 \pm .0403
3, 4 .T	0.2062 \pm .0417	0.2501 \pm .0408	0.4200 \pm .0359	0.2547 \pm .0407

Study of this table reveals a general tendency on the part of crossing over in region 1, when correlated with doubles including region 1, to rise to a high point and then to decline from this point to the end of the chromosome. The same general tendency will be seen when region 4 is correlated with the successive doubles including 4. That is, the correlation rises to 2, 4, then declines toward 1, 4. In the middle of the chromosome both regions have their high correlations at the end of region 1. Thus it

TABLE 23
Singular partial correlation coefficients for table 22 (second order).

Double	Singles, region 1 correlation	Double	Singles, region 2 correlation	Double	Singles, region 3 correlation	Double	Singles, region 4 correlation
1, 2 .T2	0.3054 \pm .0395	1, 2 .T1	0.4469 \pm .0348	1, 2 .T1	0.0934 \pm .0432	1, 2 .T1	0.0072 \pm .0435
1, 2 .T3	0.4078 \pm .0362	1, 2 .T3	0.5137 \pm .0320	1, 2 .T2	0.0914 \pm .0432	1, 2 .T2	0.0370 \pm .0435
1, 2 .T4	0.4152 \pm .0347	1, 2 .T4	0.5459 \pm .0306	1, 2 .T4	0.2351 \pm .0411	1, 2 .T3	0.0494 \pm .0434
1, 3 .T2	0.5190 \pm .0318	1, 3 .T1	0.1719 \pm .0423	1, 3 .T1	0.3124 \pm .0393	1, 3 .T1	-0.0099 \pm .0435
1, 3 .T3	0.5170 \pm .0319	1, 3 .T3	0.2709 \pm .0403	1, 3 .T2	0.3752 \pm .0374	1, 3 .T2	0.0427 \pm .0435
1, 3 .T4	0.5872 \pm .0285	1, 3 .T4	0.3624 \pm .0378	1, 3 .T4	0.4413 \pm .0351	1, 3 .T3	0.0461 \pm .0434
1, 4 .T2	0.2936 \pm .0398	1, 4 .T1	-0.0118 \pm .0435	1, 4 .T1	0.0114 \pm .0435	1, 4 .T1	0.1720 \pm .0423
1, 4 .T3	0.2902 \pm .0399	1, 4 .T3	0.0827 \pm .0433	1, 4 .T2	0.0958 \pm .0431	1, 4 .T2	0.1928 \pm .0419
1, 4 .T4	0.2997 \pm .0396	1, 4 .T4	0.1090 \pm .0435	1, 4 .T4	0.1211 \pm .0429	1, 4 .T3	0.1940 \pm .0419
2, 3 .T2	-0.0745 \pm .0433	2, 3 .T1	0.3299 \pm .0388	2, 3 .T1	0.3305 \pm .0386	2, 3 .T1	0.0241 \pm .0435
2, 3 .T3	-0.0572 \pm .0434	2, 3 .T3	0.2533 \pm .0408	2, 3 .T2	0.2662 \pm .0406	2, 3 .T2	0.0173 \pm .0435
2, 3 .T4	0.0646 \pm .0434	2, 3 .T4	0.3276 \pm .0389	2, 3 .T4	0.3375 \pm .0386	2, 3 .T3	0.0210 \pm .0435
2, 4 .T2	0.0679 \pm .0433	2, 4 .T1	0.4568 \pm .0344	2, 4 .T1	0.1061 \pm .0430	2, 4 .T1	0.2533 \pm .0407
2, 4 .T3	0.2092 \pm .0416	2, 4 .T3	0.4821 \pm .0334	2, 4 .T2	0.0417 \pm .0435	2, 4 .T2	0.2883 \pm .0399
2, 4 .T4	0.2382 \pm .0411	2, 4 .T4	0.5151 \pm .0320	2, 4 .T4	0.1837 \pm .0421	2, 4 .T3	0.2699 \pm .0404
3, 4 .T2	0.1192 \pm .0429	3, 4 .T1	0.1867 \pm .0420	3, 4 .T1	0.3795 \pm .0373	3, 4 .T1	0.2387 \pm .0411
3, 4 .T3	0.0699 \pm .0433	3, 4 .T3	0.1439 \pm .0426	3, 4 .T2	0.3737 \pm .0374	3, 4 .T2	0.2515 \pm .0408
3, 4 .T4	0.1855 \pm .0420	3, 4 .T4	0.2468 \pm .0409	3, 4 .T4	0.4256 \pm .0356	3, 4 .T3	0.2651 \pm .0405

is seen that the relation of double crossovers to the regions surrounding them forms curves, the crest of the curves occurring between 20 and 30 units.

But there are discrepancies in the high point of this curve. It will be noticed that there is a significant correlation between region 1 and the double including regions 2 and 4. In the same way, 2 is correlated with 1, 3 and 3 with 1, 4 significantly as measured by its probable error. These correlations would not be expected, and the question arises as to what they are due.

We have seen that the single crossovers in the various regions are correlated variates in which the correlation is most pronounced between adjacent regions. If, then, crossing over in region 1 has a sufficiently high correlation with crossing over in region 2, it would be expected that double crossing over including region 2 would also be correlated with region 1. To test this hypothesis for this case and the similar cases as given above, it is necessary to form the previously described universe, in which the correlation between the two continuous variables 1 and 2, 4 for a constant value of a third variable 1 and 2 is determined, where all values of the offspring are held constant. The measure of such a correlation has been termed the singular partial correlation by PEARSON (1914).

The values for the successive singular partial correlation coefficients (second order) for the above data are given in the table below in which the terms held constant are separated from the correlated term by a dot.

For the complete analysis of the problem the third-order singular partial coefficients needed are given in table 24.

TABLE 24
Singular partial correlations for table 23 (third order).

Double	Singles region 1	Double	Singles region 2
2, 3 .2T3	-0.0845 \pm .0432	1, 3 .1T3	0.1223 \pm .0429
2, 4 .2T4	0.0421 \pm .0435	1, 4 .1T4	-0.0123 \pm .0435
3, 4 .3T4	0.0442 \pm .0435	3, 4 .3T4	0.1834 \pm .0421

Double	Singles region 3	Double	Singles region 4
1, 2 .1T2	0.0124 \pm .0435	1, 2 .1T2	0.0070 \pm .0435
1, 4 .1T4	0.0124 \pm .0435	1, 3 .1T3	0.0119 \pm .0435
2, 4 .2T4	0.0371 \pm .0435	1, 4 .1T4	0.0169 \pm .0435

A study of these tables shows that where before a significant correlation was observed between singles in one region and doubles not including this region as one of the breaking segments when coefficients of the first order were used, now a significant correlation is present only when the double also includes the single as one of the breaking segments. Thus the previous hypothesis to account for these discrepancies was correct. Two double crossovers correlated with a given region may have a significant correlation with that region due to their both being correlated with a third region.

The last difficulty in the study of the data at hand for the purpose of determining how double crossovers are related to the various regions of the chromosome is removed. The correlations of table 23 show that double crossing over, including region 1, is not distributed at random, but is more apt to have a second simultaneous break in region 3 than in any other, the difference in this case running as high as seventeen times the probable error.

Each of the end regions exhibit that rise in the relationship in the middle of the chromosome which, as has been previously pointed out in the first part of the paper, would be expected on the basis of the twisting hypothesis where there was a definite ratio of twist. These waves rise rather sharply to the mid-point and drop off rapidly in the other direction. The relations of region 1 to double crossing over including the other successive regions is that best suited to bring out this rise, for, as has been previously pointed out, the region 1 is so short that twists of the same length cannot extend into either of two regions. This, then, forms the best test of the hypothesis of twisting to account for crossing over versus any other hypothesis which calls for the distribution of crossovers at random. Region 1 correlated with the successive doubles rises sharply to 3 and falls rapidly to 4. Region 4 rises to 2 and falls to 1. The rise and fall in 4 is less rapid, due to its being so long a segment that it enables a twist falling within its bounds to fall in either of two regions, depending on whether or not its first break is near one end or the other, still even in this the mode is marked. The mid-regions also exhibit a rise toward the ends as would be expected, although as the number of factors is too few, no mode appears. This high point and this drop may then represent a twisting taking place about every 20 to 30 units in the third chromosome.

If these correlations are considered from the point of view of the reduction theory to account for the interchange, what is it necessary to consider? Not only does the rise and fall of the correlation have to be

taken into account but also account has to be taken of the whole correlation, for no correlation can be expected on the reduplication theory as previously shown. Correlations have then to consider nothing, even when ranging from twenty-four times the probable error to those four times the probable error. The odds against this being accidental are enormous. It may then be said that the twisting hypothesis for crossing over accounts for the facts remarkably well, while the reduplication theory accounts for them not at all. Consequently, the experimental facts here deduced in carefully controlled and analyzed experiments indicate crossing over to take place between loosely twisted, finely spun-out chromosome threads with between 25 and 30 units as the modal distance between successive crossovers. Because of the uneven intervals and the inaccuracy of the moments calculated from them due to the few classes on which they would have to be based, it is not the purpose of this paper to treat the frequencies other than by the use of correlation coefficients. It is, however, of interest to try the moment calculation for the position of mode for the best suited of these classes for analysis. The approximate ratio between the two twists when calculated for the s_6D' region by the formula:

position of the mode = mean — $\frac{1}{2} \frac{1\mu_3(r-2)15}{2\mu_2(4+2)}$, is shown to be at

26.24 units, considering each class as distributed around the mid-ordinates (an hypothesis obviously untrue, but giving the best approximation to the true value which it is possible to obtain since the true mean of each class is not known). The use of the above formula for the mean is justified, as the curve is shown to be type IV by $\beta_1=+.057$ and $\beta_2=+.68.313$.

DISCUSSION

The geometrical interpretation put upon the rise and fall of the double-crossover frequencies may seem rather speculative in character. It is, however, I venture to think, supported by a good deal of strong evidence. Since the idea that the chromosomes are the bearers of the determiners of hereditary characters was put forward by WEISMANN and ROUX and applied to Mendelian inheritance by SUTTON, there has been an ever increasing amount of evidence collected that it is to the chromosome we must look for the mechanism of heredity. As a basis for this conclusion the studies of STEVENS and WILSON have shown the parallel between sex and the behavior of a chromosome pair. This was followed by the work of MORGAN, showing that this parallel included the so-called sex-linked factors as well as sex. As direct evidence, we may draw first on that of BOVERI on multipolar mitosis, of BALTZER on reciprocal crosses of sea-

urchins and that of HERBST (1909) and GODLEWSKI (1911) on parthenogenesis and fertilization. Further evidence comes from the work of LUTZ (1912), GEERTS (1911) and GATES (1907, *et seq.*) in the study of *Oenothera* mutants. With all, perhaps the most brilliant piece of evidence is that of BRIDGES (1916) where proof is given that the sex chromosomes bear the sex-linked factors; for here by cytology and genetics he can follow the course of the sex-chromosomes and of the factors carried thereby. These names do not exhaust the list of those who have added materially to the proof that the chromosomes are the bearers of the hereditary characters, yet it seems to me that these constitute as complete a chain of crucial experimental evidence as would be required by the most rigorous logic, consequently the conclusion will not be crowded by presenting more. Should it be granted, however, that this does constitute proof, it requires that all hypotheses to account for the interchange of linked or coupled factors shall rest on the chromosome; it requires that the reduplication hypothesis shall segregate its genetic ratios through the agency of the chromosomes.

If other evidence is taken purely from the experimental side of genetics and consideration be given to STURTEVANT'S (1914) criticism, the reduplication hypothesis in what TROW (1912) and BAILEY (1913) have shown to be the general mathematical relations of its gametes, it is found that it is doubtful if reduplication is able to explain even the ratios that are obtained. Thus in the case of TROW'S special hypothesis STURTEVANT shows that in every case the calculated is greater than the observed ratios. The difference is significant in every case and in the same direction. Further, STURTEVANT shows that if the general hypothesis is used the number of cell divisions required are at hopeless variance when considered with the possible divisions. It may then be said that deduction from the theory leads to a poor agreement between this theory and fact.

The students of genetics who use the linkage hypothesis to explain their ratios have some evidence to show that the linkage hypothesis is also applicable to the same forms on which the reduplication hypothesis is based. BRIDGES (1914) has shown that in the experiments of PUNNETT (1913) on sweet peas and GREGORY (1911) on *Primula* the linkage hypothesis is at least applicable. Unfortunately, BRIDGES used as his measure of linkage the coefficient of 'association' of YULE, which is in itself of rather doubtful value as a measure of relationship, as has been shown by HERON (1911), and HERON and PEARSON (1913). Fortunately, however, the conclusions of BRIDGES have been justified, since we now have some very excellent data presented by ALTENBURG (1916)

in evidence that the linkage hypothesis can be applied to *Primula*. Data on some 3600 plants show clearly that it is exceedingly difficult to see how a reduplicating series can be made to fit.

ALTENBURG separates his cases in which the male plants were heterozygous and the cases in which his female plants were heterozygous. There seemed to be quite a difference in the amount of crossing over between them, so I thought it might pay to test each of the classes for the likelihood of this coming from random sampling. The table 25 shows the result of this test.

TABLE 25

	♂ Hete- rozygous	♀ Hete- rozygous	Percent ♂	Percent ♀	Difference	Diff. ÷ P.E. Diff.
Non-crossovers	1829	266	55.558929	67.807132	12.298 ± 1.716	7.2
First single	1053	107	31.986633	27.295914	4.691 ± 1.437	3.2
Second single	325	11	9.872418	2.806122	7.066 ± 0.956	7.4
Double	85*	8*				

* Not calculated, as the value of q would be very small.

Thus it is seen that the difference is well above three times the probable error. The range of probability that these differences came from random sampling are 31 to 1 for the 3.2 times the probable error to 1,675,321 to 1 for the 7.2 times the probable error. This difference certainly looks significant. Since all of these plants were raised under the same conditions and cared for alike, it would seem that crossing over in the female is less than that in the male due to some differential effect of the sex. Such a graded effect, taken in connection with the other known facts for crossing over, indicates that when a sufficient number of animals and plants are known, a graded series of crossing-over values may be found, extending from *Drosophila* with crossing over only in the female, through sweet pea and *Primula* with crossing over in both sexes, to silk-worms and probably chickens, with crossing over only in the male. Such a series would then duplicate the series found for the Y chromosomes, although it would not parallel it.

To return to our general theme, in discussing this paper of ALTENBURG (1916), PUNNETT (1917) suggests that the reduplication hypothesis calls for a marked difference for the reduplicating series for the *BC*

regions in the back crosses, $\frac{BAC}{bac} \times \frac{bac}{bac}$ and $\frac{BC}{bc} \times \frac{bc}{bc}$.⁴

⁴ "On the chromosome hypothesis there is only one set of positions which allows of:

At first sight the difference in crossing over shown when different genes are interpolated between two fixed genes would seem to agree with this expectation for the reduplication hypothesis as above stated. In non-conformity with the requirements of this hypothesis as stated by PUNNETT, the total crossing over may be significantly increased by the presence of *A* heterozygous and be diminished in a like case where another *A* is heterozygous as compared with the crossing over of the $\frac{BC}{bc} \times \frac{bc}{bc}$ condition. Further, the change of a factor outside the region *BC* may affect the crossing over of that region more than a similar change of a factor within the region itself. Let us now take the data in the tables and arrange two cases to conform with the needs of the reduplication hypothesis. There are several possible cases of this kind which could be made. The choice of the particular case seems immaterial, consequently let us confine attention to the back-crossed females heterozygous for sepia, spineless on one side, dichaete on the other, and females heterozygous for the genes sepia and spineless. For this table the data from appendix tables A, D, and C are available. The data collected and so reduced are given in tables 26 and 27.

two of the coupling values between three factors *A*, *B* and *C* to be equal, viz., when two loci are equidistant from the third, thus:

B *A* *C*

The coupling or linkage values between *A* and *B*, and between *A* and *C*, are here of the same value, but when this is so it follows of necessity that the value for *B* and *C* must be considerably lower than either of the other two. If a three-factor case were found of such a nature that two of the values were equal and the third definitely higher, such a case might serve as a criterion between the two hypotheses." Further, he says, "Such a case is probably to be found among *Primulas* in connection with the three pairs of characters, magenta (*M*) and red (*r*), short style (*S*) and long style (*s*), green stigma (*G*) and red stigma (*g*)."
GREGORY and ALTENBURG have both published on these, but, as PUNNETT, says, "The figures" (ALTENBURG's) "as they stand offer of course no criterion between the rival hypotheses, for the critical experiment is yet to be made. This consists in the cross between *SsGg* plants (ex *SG* × *sg*) and the double recessive *ssgg*, where all individuals used are homozygous for either *M* or *m*. On the chromosome hypothesis the linkage values should remain the same as those given above (where *M* is present in heterozygous forms); on the reduplication hypothesis we should expect to find the linkage higher, probably of the form 2*SG*: 1*Sg*: 1*sG*: 2*sg*." This statement has several obscure points more especially as to how this comparison of the two distributions is to be made. If, as the text would indicate, the comparison is to be between the reduplication series as applied to each set of data, instead of comparing the actual distribution of the data, the reasoning is in error for, as STURTEVANT has shown that the series obtained on Trow's hypothesis, always is significantly too high. In our comparison we shall, therefore, consider only the actual figures.

TABLE 26

	D'	$s_o s_n$	$s_e D' s_n$	s_n	$D' s_n$	s_o	$s_e D' s_n$	N
Table A	3532	3257	381	368	244	307	27	26
Table D	12758	12357	1666	1583	1260	1507	145	196

For table C when dichæte is not present :

TABLE 27

	Non-crossovers		Single crossovers	
	$s_e s_n$	N	s_o	s_n
Table C	968	1153	277	233

Considering only the series $s_e s_n$ which, according to PUNNETT's statement of the reduplication hypothesis, the series of A and D should differ from that of C we have :

TABLE 28

	$s_e s_n$	s_e	s_n	N
Table A	3284	688	612	3558
Best fitting series 5:1:1:5	3395	679	679	3395
Table D	12502	3173	2843	12954
Best fitting series 4:1:1:4	12589	3147	3147	12589
Table C	968	277	233	1153
Best fitting series 4:1:1:4	1052	263	263	1052

None of these expected series agrees with the actual series as well as could be wished. Thus table A could be best fitted by a series of about 4.6 to 1. Table C would have a better agreement between actual and expected, fitted with a series of 4.4 to 1. This is a fundamental drawback to the theory of reduplication, for the search for simple series often obscures real differences. Thus the *Primula* series treated by PUNNETT (1917) leads to a theoretical distribution on TROW's hypothesis of secondary reduplication which could have the actual distribution observed in the *SG* series selected from it in samples of 3684 individuals each in not more than 1 in 2500 such samples. Yet the uncritical nature of the hypothesis led PUNNETT to conclude that the result is in fair accord with expectation. Thus in our experiment there are significant differences in the distributions taken as a whole, but these differences follow no rule.

Comparing the reduplicating series above, it is seen that they lead to practically the same thing; that is, 4:1:1:4, instead of coming to markedly different series as the reduplication hypothesis calls for. The conclusion seems forced upon us by this test of the hypothesis that the facts of the several factor crosses do not agree with this hypothesis, but that the twisting hypothesis, based as it is on the known chromosome behavior, does fit the facts.

The conclusion that the reduplication hypothesis does not explain the several factor cases is further borne out by the fundamental experiments of PLOUGH (1917). In this investigation the temperature effect on crossing-over rate enabled him to show that crossing over does not occur in the early oögonial divisions, and there is good reason to believe that the percentage of crossing over is affected by temperature only in the growth period of the egg. Thus the long series of differential cell divisions necessary for the formation of the reduplicating series is shown to be absent in actual point of fact.

Adopting the explanation that it is the chromosomes that must be looked to for the mechanism of crossing over, the inquiry may be made as to how crossing over is brought about. STURTEVANT (1913) has shown that it is possible to map the position of the factors in the chromosome by crossing-over ratios. This principle has been extended and has been shown to be applicable to all the *Drosophila* chromosomes. The practical value of the hypothesis may thus be said to be proved, and through the work of MORGAN, STURTEVANT, BRIDGES and MULLER (1915) all of the chromosomes are mapped. As was pointed out in a preceding section of this paper, the ratios may vary, yet in no case does this variation affect the relative position of the factors.

It becomes important, then, to inquire how this exchange takes place. The function of this paper is a specific inquiry into what the ratios of the double crossovers to the different single crossover regions would show as to this interchange. Following the ideas of JANSSENS (1909) as elaborated by MORGAN (1916), MULLER, BRIDGES, and PLOUGH (1917), of a twisting of the chromosomes, although considering that this twisting takes place at an earlier stage and between the finer threads of the chromosomes, it has been possible to show that the results are what would be expected on the twisting hypothesis to account for crossing over. Turning more to the general aspects of the case, it is seen that this gives a strong foundation for a single mechanical explanation for the exchange of factors where there is only one scheme to account for the whole. It is not known why a fusion should take place where it

does or why the genes should line up along the chromosomes as accurately as they do. These are problems of the future, but they do not in any way influence the fact that the hypothesis of loose twisting explains the observed ratios for the third chromosome in so many of its intricacies, as to carry the conviction that it is much more than just a chance relationship.

In conclusion, I wish to express my thanks to Prof. E. B. WILSON and Dr. C. B. DAVENPORT for opportunities accorded me for research; to Prof. T. H. MORGAN, Dr. A. H. STURTEVANT, and Dr. C. B. BRIDGES for their ever-ready assistance and suggestion, and to Dr. RAYMOND PEARL for his constant interest in my work.

SUMMARY

This paper is a contribution toward the analysis of the normal fluctuating variations in crossing over as seen in the third chromosome of *Drosophila melanogaster*. The third chromosomes of each female, as shown by her offspring, cross over a certain number of times. The variability is studied by comparing the results from different females. The association between the crossovers is studied by comparing the results within each given female.

1. The means, standard deviations, and the coefficients of variation are given for the distributions of each region in this chromosome under discussion. The features of chief interest are the great variability of both single crossing over and double crossing over. The coefficient of variability ranges between 18 and 59 for the single crossovers and 67 and 110 for the double crossovers. The actual amount of this coefficient is apparently dependent to some degree on the actual mean size of the crossing-over ratio.

2. A table is presented to show the relative variability of crossing over in comparison with that of other physiological and morphological characters. The table shows that crossing over is one of the most highly variable phenomena known, indicating that the mechanism behind crossing over is not as precise as that found in most physiological studies.

3. As a necessary preface to the analysis of the internal mechanism of variations of crossing over it has been pointed out that it is essential to know how much the ratios are influenced by external agencies. Toward this end it has been shown (page 214) that to some degree the absolute value of the crossing-over ratio varies according to the genes present in the chromosome. Further, it is shown that no significant

effect on crossing over was produced by the food or temperature used or by the variations of season or bottle output.

4. Since it had been shown that the crossing-over values are influenced by the known genes present, it became essential to know whether or not there were any modifying genes present influencing the ratios. A selection experiment was performed to test this. The parent and offspring correlations for this experiment ranged from $+ 0.336 \pm .135$ to $-.0211 \pm .118$ for the selection for low crossing over; for high crossing over from $+ 0.150 \pm .248$ to $- 0.134 \pm .191$. The conclusion is to be drawn that there were no differences in modifying factors for crossing over in the experiment.

5. In the resolution of the single crossing-over ratios into their component elements it was shown that there is a significant correlation between the crossing over in different regions. In general this difference progresses from the left-hand end of the chromosome to the right. Thus the correlation between region 1 and 2 is $+ 0.4019 \pm .0365$, between 1 and 3 is $+ 0.3492 \pm .0382$, and between 1 and 4 is $+ 0.1039 \pm .0430$. The explanation of this difference is obscure.

6. A relationship between single and double crossing over is shown to exist, such that a crossover in one region is more likely to be accompanied by another simultaneous crossing over in a region 25 to 35 units away than it is to be accompanied by a simultaneous crossing over in any other region. Thus when region 1 is correlated with its double crossover including regions 2, 3, and 4, respectively, the correlations are $+ 0.3054 \pm .0395$, $+ 0.5170 \pm .0319$, and $+ 0.2997 \pm .0396$. This rise and fall, together with a definite mode, is held to mean that there is a modal interval between two successive crossovers. Thus the two finely spun-out chromosomes, when they come together prior to crossing over, apparently twist about each other loosely and generally have the points of contact where breaking may take place about 25 to 30 units apart.

APPENDIX

TABLE B

$$\text{Cross: } \frac{D' \quad p'' \quad s_s \quad e'' \quad r_o}{N} \times \delta \quad \frac{p'' \quad s_s \quad e'' \quad r_o}{p'' \quad s_s \quad e'' \quad r_o}$$

References	0	1	2	3	4	1, 2	1, 3	1, 4	2, 3	2, 4	3, 4	1,2,3	1,2,4	1,3,4	Total	
627	46	59	3	3	16	12	17	14	19	9	1	1	2	2	2	214
629	45	41	9	9	8	12	13	15	17	6	1	1	3	1	2	186
632	29	34	2	8	17	9	15	3	11	7	1	1	2	1	2	149
634	47	57	2	2	14	15	15	18	19	20	3	1	2	1	1	220
658	42	45	1	1	4	3	4	11	11	9	1	2	1	1	2	138
665	78	90	6	9	24	21	24	27	21	26	2	3	4	3	4	353
665b	51	64	2	5	13	15	16	11	17	16	1	1	1	1	1	216
665c	42	52	5	2	10	7	8	24	11	1	1	2	3	1	1	168
700c	22	27	1	1	4	3	4	3	9	5	1	1	1	1	1	80
706	25	18	2	2	1	4	7	6	8	1	1	1	1	1	1	74
708a	23	31	5	2	9	6	11	10	9	7	1	2	1	1	2	123
708b	15	25	2	1	4	3	5	5	5	6	1	1	1	1	1	71
708c	27	39	2	1	5	8	7	7	10	10	1	1	1	1	1	117
710	15	22	2	2	4	4	14	4	1	1	1	1	1	1	1	66
718b	23	34	1	2	8	8	6	9	8	5	1	1	1	4	1	111
728a	25	27	4	1	2	3	3	5	12	5	1	2	2	2	1	95
Total	1220	92	263	312	366	15	25	30	9	32	11	1	4	1	2381	

TABLE C

$$\text{Cross: } \frac{s_o \quad s_s \quad c'' \quad r_o}{H'} \times \delta \quad \frac{s_o \quad s_s \quad c'' \quad r_o}{s_o \quad s_s \quad c'' \quad r_o}$$

References	0	1	2	3	4	1, 2	1, 3	1, 4	2, 3	2, 4	3, 4	1,2,3	1,2,4	Total
1052	42	20	8	15	3	6	1	7	6	2	2	1	1	115
1157	56	55	6	13	5	10	1	9	16	1	1	5	1	183
1161	55	45	7	7	3	20	21	1	1	1	6	1	1	168
1162	57	51	20	12	10	6	1	18	18	1	3	1	1	199
1163	54	40	9	9	2	6	1	6	7	1	2	1	1	136
1189	60	58	18	14	10	5	3	19	18	1	1	4	7	217
1192	38	28	14	8	4	2	1	12	13	1	1	5	5	137
1193	58	66	21	18	7	14	2	25	17	1	2	3	8	244
1194	39	31	13	13	2	4	1	8	12	1	1	2	5	132
1195	66	40	12	10	4	15	1	23	24	3	1	3	1	212
1197	87	58	21	11	9	9	1	24	18	1	1	2	3	247
1220	17	20	7	2	2	1	1	2	2	2	1	1	1	58
1242	18	16	5	2	1	1	1	2	3	1	1	1	1	48
1249	39	27	13	9	4	4	1	10	15	1	1	3	1	127
1250	38	29	8	9	5	1	1	7	2	3	2	1	1	107
1251	27	21	12	8	5	2	2	7	6	1	1	3	1	95
1270	12	12	1	1	1	3	1	8	5	1	1	3	1	48
1273	15	15	7	6	2	2	1	4	6	1	1	2	1	61
1280	15	13	1	3	2	3	1	9	7	1	2	1	1	56
1275	10	11	2	4	1	1	1	6	1	1	1	2	1	41
Total	1459	378	175	22	443	31	8	89	10	10	2	3	1	2631

Refer- ences	0	1	2	3	2,6	1,3,4	1,3,6	1,4,6	2,3,6	3,4,6	3,5,6	Total
1348	112	78	12	9	5	3	9	2	.	.	.	324
1349	104	97	12	11	1	.	10	10	.	.	.	360
1350	111	113	11	12	3	2	4	5	.	.	.	379
1351	107	86	13	9	1	1	6	5	.	.	.	339
1352	116	113	12	8	4	1	3	2	.	.	.	350
1363	66	57	13	3	2	1	1	2	.	.	.	209
1364	38	49	4	4	.	1	4	3	.	.	.	140
1365	78	77	11	13	4	1	6	8	.	.	.	276
1366	63	76	9	13	2	.	6	9	.	.	.	265
1368	79	69	4	6	.	.	4	3	.	.	.	243
1369	74	60	10	12	6	1	2	2	.	.	.	259
1372	51	44	8	6	2	2	4	3	.	.	.	185
1382	58	69	6	7	.	3	6	4	.	.	.	228
1383	50	52	6	6	2	2	6	2	.	.	.	209
1385	27	26	6	2	1	.	5	6	.	.	.	125
1386	72	76	3	3	.	.	4	2	.	.	.	228
1387	70	65	12	9	2	.	7	2	.	.	.	231
1388	69	54	7	5	4	.	.	6	.	.	.	227
1389	59	61	8	11	1	2	7	5	.	.	.	227
1390	77	93	12	8	1	2	3	4	.	.	.	275
1391	49	35	5	11	2	3	6	3	.	.	.	167
1392	75	67	6	8	2	3	4	5	.	.	.	245
1393	72	64	2	6	1	2	2	7	.	.	.	210
1395	36	23	.	2	.	1	3	82
1396	53	40	5	8	3	1	4	4	.	.	.	181
1397	40	46	6	8	2	.	2	3	.	.	.	154
1415	62	54	10	1	3	.	4	3	.	.	.	193
1417	71	58	8	6	2	5	10	8	.	.	1	261
1420	67	73	5	12	4	.	8	1	.	.	.	243
1422	79	72	11	7	1	4	6	5	.	.	.	275
1426	60	49	11	9	1	.	6	3	.	.	.	221
1427	53	60	8	3	2	.	7	4	.	.	.	215
1428	54	20	.	4	.	.	4	4	.	.	.	105
1429	27	29	7	4	2	.	6	2	.	.	.	113
1431	22	12	2	1	.	1	2	1	.	.	.	55
1432	75	62	5	5	1	1	1	5	.	.	.	211
1473	45	44	7	5	2	.	2	2	.	.	.	157

TABLE D

$$\text{Cross: } \frac{s_a \quad c^a \quad r_a}{D'} \times \delta \quad \frac{s_a \quad s_b \quad c^b \quad r_a}{s_a \quad s_b \quad c^b \quad r_a}$$

References	0	1	2	3	4	1,2	1,3	1,4	2,3	2,4	3,4	1,2,3	1,2,4	1,3,4	2,3,4	1,2,3,4	Total
588	27 25	5 5	6 3	10 4	8 2												97
666	14 14	2 2	3 4	4 5	2 2	3	1			1	4						60
737	8 12	3 1	1 1	2 5	8 2			1									44
740	53 63	7 14	10 10	6 17	26 28	3 2	1 3	3 3	1	1 2	1						244
741	4 6		1 1	1 1	1 3			1		1							19
747	53 47	8 10	9 9	6 6	23 25	1	4 3	1		1 5	2						213
748	11 4	1 2	1 2	1 3	4 4	1			1	1							36
750	43 36	4 5	3 2	7 16	7 1	2	1 2	1 2	1	2 1							139
751	55 20	1 2	2 4	8 6				2	1	1							102
754	74 66	3 2	1 2	3 6	1						1						45
756	15 11	4 7	3 8	10 7	28 24		1 1	1 1	1	1	2						238
757	26 29	4 2	4 6	2 4	9 6			1	1								94
758	6 6	2 1	2 1	1 1				1									20
759	63 63	6 6	1 6	7 6	25 29	2	4 2	3 1	1	2 1							228
760	44 49	5 3	2 7	4 5	17 9	1	1 2	3	1	1			2				156
761	41 41	5 7	2 2	6 22	20 1			2									149
762	60 57	6 7	2 4	9 5	20 18	1		2		1 1							193
763	52 53	0 5	3 4	10 7	14 12	1 1	1	3 4	1	1 1							171
764	40 35	5 3	4 1	8 5	6 12	1	1	3		2							125
766	38 40	4 4	6 3	3 12	10 1	2	3	2 2		1 1							132
767	27 35	4 5	5 5	3 2	16 7												109
770	14 19	2 2	1 3	1 2	6		1 1										52
772	9 10	1 1	1 2		1					1							26
774	28 18	2 3	1 2	1 4	10 9		1 3		1								83
775	52 20	4 7	1 2	1 3	2 10	1	1 1										105
776	38 31	2 3	7 9	5 4	5 12	2 1	2 1	1	1	1 1			1				127
777	22 24	11 2	1 3	7 2	14 8			1 1	1 1	1							99
778	25 20	2 4	7 9	4 4	8 18	3 2	1 3	2	2	2							116
779	40 48	1 1	6 6	8 7	15 18		1 4	2 1	1 1	1							160
780	31 31	2 2	3 4	4 5	4 4					1							91
781	27 28	2 9	1 4	6 14	13 1			4	3								112
782	36 29	5 1	4 8	1 3	14 13	1	3	2 1	1	3 2							127
783	64 62	2 8	7 5	13 14	15 26	1	4	4	1	1 1				1	1		240
784	58 64	6 6	7 2	6 4	10 11		1 1	1 1	1 2	2			1			1	184
785	29 32	5 2	2 1	8 10			1 1	2									93
786	44 36	3 3	7 9	4 2	7 9	1		1		2	1			1			130
788	40 46	6 5	5 9	8 7	9 11	2 2	1 2	4 3	2	4 2							168
789	52 39	5 4	4 6	8 7	11 14	2 1	3 3	3 3	1	2 2	1		1 1				170
790	107 85	2 5	6 7	5 9	20 22	1	1 1	1 4		1 1	1			1			290
791	52 51	8 6	5 1	5 14	14 1	1	1	1 6		1 1	1						164
793	28 29	5 1	3 4	5 6	9 21			1 1		1 1							115
795	30 49	4 9	8 13	15 6	13 14	1	2 2	5 1	1	2	1			1			187
797	26 28	3 3	8 5	6 6	11 1	1	2		1	1			1		1		113
799	16 10	1 3	2 1	1 1	2 1	1											39
800	43 59	3 2	2 7	3 9	9 15		1 1	1 2		1 1	1						159
901	34 43	3 5	6 1	4 4	14 12		1	4		2 1	1						135
902	47 57	3 6	2 3	8 7	20 15	3	1	1 2									175
903	13 10	2 1	3 1	1 4	4 4			1 1	1								42
904	23 31	3 2	3 2	2 4	12 4			1									87
905	65 55	7 4	5 7	8 5	22 21	1	2 3	4		2			1				212
906	45 34	13 5	9 5	16 4	14 11		1	1 1	1		3 1						164
907	52 50	2 9	8 13	7 8	15 17	2	1 2	3 2		2 1	1			1		1	207
908	57 50	6 7	5 7	3 4	7 15		2	5		2							170
909	37 48	4 7	5 4	9 11	18 15	2	6 2	3 5	1	5 1	1						195
910	45 44	7 4	6 6	5 5	9 11	1	2	1 2	1 1	1							151
911	35 34	3 2	3 6	3 5	11 7	1	2 3	2		1							119
912	27 31	4 5	2 2	1 3	3 6		1 1	2		3					1		92
913	19 19	5 4	2 6	1 3	10 10	1	1	1 1		1 1				1	1		88
914	13 19	2 2	3	1 2	12 8	1											63

TABLE D (continued)

[illegible]

TABLE D (continued)

References	0	1	2	3	4	1, 2	1, 3	1, 4	2, 3	2, 4	3, 4	1, 2, 3	1, 2, 4	1, 3, 4	2, 3, 4	1, 2, 3, 4	Total
996	21 24	3 4	4 4	6	10 11	1	1	1	1	1	1	1	1	1	1	1	93
997	29 28	2 1	8 6	3 3	7 3	2	1	1	1	1	1	1	1	1	1	1	98
998	26 26	3	2 1	1 8	14 9	1	1	2	1	1	1	1	1	1	1	1	94
999	24 27	4 7	2 5	1 4	6 5	1 1	1	1	1	1	1	1	1	1	1	1	87
1002	17 11	2 5	2 1	1 4	6 6	1	1	2	1	1	1	1	1	1	1	1	59
1003	29 13	2 2	1 3	4 5	5 5	1 2	1 6	1	1	1	1	1	1	1	1	1	80
1005	26 20	2 3	4 2	3 4	8 6	1	1	1	1	3	1	1	1	1	1	1	83
1010	10 16	3 1	3 4	7	1 6	1	1	1	2	1	1	1	1	1	1	1	54
1011	13 17	3 4	2 1	3 2	8 3	1	2	1	1	2	1	1	1	1	1	1	61
1014	26 19	1	1 4	2 1	7 7	1	1	1	1	1	1	1	1	1	1	1	70
1023	14 22	4 3	2 4	3	5 5	2	1	2	1	1	1	1	1	1	1	1	67
1025	18 20	1 4	2 6	2 4	11 7	1 1	1	1 1	1 1	1 1	1	1	1	1	1	1	82
1026	53 51	5 4	7 5	8 5	12 13	1 1	1	2 2	1 1	1 1	1	1	1	1	1	1	172
1027	47 41	5 2	7 4	5 8	8 7	1	1	1	1	1	1	1	1	1	1	1	139
1028	28 51	9 5	6 9	7 9	18 10	4 2	1 1	2 2	1	1	1	1	1	1	1	1	167
1029	40 35	5 6	5 2	9 10	11 9	2	1	2 2	1	1	1	2 1	1	1	1	1	146
1031	33 32	9 7	13 12	8 11	17 11	1 1	1 3	2	1	4 2	1 1	1	1	1	1	1	173
1032	33 45	10 9	13 11	8 5	14 16	1 1	3 3	3 1	1	3 2	1 1	1	1	1	1	1	180
1033	56 60	4 4	3 2	9 11	22 17	1	1	6 3	1	1	1	1	1	1	1	1	201
1034	35 49	9 11	4 1	4 10	14 16	1	3 1	1	1	1 1	1	1	1	1	1	1	162
1035	33 23	2 2	2 5	6 7	10 6	2	1	1 3	1	1	1	1	1	1	1	1	104
1036	58 52	4 4	9 8	6 8	7 13	2 1	1 2	2 3	2	2	1	1	1	1	1	1	185
1038	43 37	5 6	2 2	8 12	12 13	1	1	4	1	2	1	1	1	1	1	1	148
1040	37 37	4 5	2 6	7 12	7 16	1	1	3 1	1	1	1	1	1	1	1	1	140
1041	46 41	9 4	4 2	8 10	9 14	2	1	1	1	1	1	1	1	1	1	1	152
1042	35 48	5 2	6 4	6 4	1 20	1	1	1	1	3 1	1	1	1	1	1	1	137
1043	18 15	1 1	2 2	1 3	4 4	1 1	1	1	1	1	1	1	1	1	1	1	55
1046	28 35	6 4	2 7	10 6	4 3	1	2	3 2	1	1	1	1	1	1	1	1	114
1060	13 14	4 2	5 3	2	4 4	1	1	1 1	1 1	3 2	1	1	1	1	1	1	63
1063	25 43	6 4	4 12	4 8	2 6	1 2	4 2	1 3	1	1	1	1	1	1	1	1	128
1066	42 23	4 3	1 4	1 12	8	1	1	1	1	1	1	1	1	1	1	1	107
1071	46 50	5 4	3 4	5 7	17 21	1	1	3 1	1	3 3	1	1	1	1	1	1	174
1072	42 49	7 7	5 6	9 9	15 13	1	1	3 1	1	1 3	1	1	1	1	1	1	171
1075	63 56	13 8	7 5	5 8	17 23	5 5	2 1	2 1	1 1	1 1	2 1	1	1	1	1	1	228
1081	50 21	1 4	3 3	1 1	8 6	1	1	3	1	1	1	1	1	1	1	1	102
1088	68 50	7 7	7 3	10 9	15 21	2	1	1	3	1	1	1	1	1	1	1	214
1090	54 36	2 4	5 1	1 4	17 9	1	1	2	3	1	1	1	1	1	1	1	140
1093	48 35	4 3	5 8	7 6	15 9	1	1	1	2	1	1	1	1	1	1	1	147
1094	38 43	10 5	6 11	5 9	9 8	1	2 2	3 1	1	1 1	2	1	1	1	1	1	159
1095	56 58	4 8	8 5	7 5	11 8	5	1 1	2 2	1	4 1	1	1	1	1	1	1	184
1097	40 23	5 4	4	5 7	8 14	1	1 1	1	1	1	1	1	1	1	1	1	114
1101	82 61	12 7	9 10	11 18	29 15	2 1	4 5	2	1	2	1	1	1	1	1	1	272
1102	46 58	5 8	3 3	13 7	13 9	1	1	2 2	1 1	1	3	1	1	1	1	1	175
1103	66 67	13 8	4 12	11 13	31 18	1	1	1	3	1	1	1	1	1	1	1	249
1108	48 47	3 1	1 3	1 9	7 19	1	1	2	1	1	4	1	1	1	1	1	148
1109	52 51	7 5	8 9	8 10	23 19	2 1	3 1	3	1	1 1	1 1	1	1	1	1	1	208
1112	43 31	5 2	6 4	7 4	6 5	1	2	1 1	1	1	1	1	1	1	1	1	119
1113	80 49	3 1	3 5	7 10	15 15	1	1	3 5	1	1	1	1	1	1	1	1	198
1116	48 47	2 2	5 6	7 11	19 15	1	1	1 1	1	1 1	1	1	1	1	1	1	170
1118	46 46	7 6	9 7	9 9	27 28	1	3 2	3 2	1	1 2	1	1	1	1	1	1	210
1119	28 36	3 8	4 3	3 6	7 8	1	1	2 2	1	1	1	1	1	1	1	1	113
1120	51 25	8 5	9 10	5 10	12 8	1 3	1	1	2	1	1	1	1	1	1	1	151
1121	68 70	6 9	4 13	10 6	18 14	1	2 2	2	1	1	1	1	1	1	1	1	229
1131	86 95	4 17	7 11	13 15	32 34	2	1	2 1	1	2 4	2	1	1	1	1	1	328
1133	81 00	17 12	11 10	16 13	31 31	3	3 3	3 3	1	5 4	1	1	1	1	1	1	337
1139	57 61	9 7	2	1	8 14	18 9	1	2	1	2	1	1	1	1	1	1	193
1141	31 26	6 5	4	6 4	8 10	1	1 2	1	1	2	1	1	1	1	1	1	107
1142	21 46	8 6	3 6	5 8	11 14	3	1 1	1 1	1 2	2 1	1	1	1	1	1	1	143
1148	38 37	6 1	1 3	8 9	10 11	1	1	1	1	1	1	1	1	1	1	1	128

TABLE D (continued)

References	0	1	2	3	4	1,2	1,3	1,4	2,3	2,4	3,4	1,2,3	1,2,4	1,3,4	2,3,4	1,2,3,4	Total
1150	30	50	1	4	4	5	10	6	15	14	1	1	1	2	3	1	150
1151	28	30	2	1	3	4	7	6	9	4	1	1	1	1	1	1	99
1152	71	60	6	7	6	8	17	10	14	18	1	1	2	3	1	1	231
1153	63	86	11	10	7	10	12	15	22	15	1	1	2	2	2	1	264
1154	44	45	6	10	4	3	8	12	15	14	2	2	2	4	1	2	170
1156	58	52	8	8	8	5	9	7	15	22	1	1	1	1	1	1	199
1158	33	18	2	3	5	5	6	8	5	5	1	1	1	1	1	1	85
1160	45	52	14	5	1	2	5	10	15	14	2	2	1	3	1	1	170
1165	32	16	1	1	5	5	15	1	11	11	1	1	1	1	2	2	112
1169	26	19	3	2	3	5	6	8	5	5	1	1	1	3	1	1	95
1173	63	53	10	11	1	7	4	12	20	20	2	3	3	4	1	1	214
1174	80	73	10	8	3	11	16	14	25	35	4	3	5	4	4	1	105
1177	18	16	3	3	1	3	2	1	7	2	1	1	1	1	1	1	59
1179	40	41	5	4	4	8	7	4	15	8	1	1	1	3	2	2	144
1181	107	98	15	0	11	17	12	20	18	32	3	3	4	6	4	1	364
1182	95	106	12	15	15	16	16	19	25	26	1	5	4	2	4	1	365
1187	51	51	1	8	3	8	5	6	14	9	1	1	1	3	3	1	169
1188	39	24	2	2	8	4	2	4	12	9	1	1	1	5	1	1	117
1199	46	39	6	5	8	12	8	15	25	10	1	1	1	3	1	1	192
1200	40	31	4	4	5	5	10	9	9	8	1	1	1	2	1	1	138
1201	34	25	4	6	6	3	2	3	9	15	1	1	1	2	1	1	113
1204	27	14	1	3	1	3	1	2	2	2	1	1	1	1	1	1	55
1206	21	16	5	4	3	2	4	2	3	12	1	1	1	1	1	1	73
1219	9	14	4	1	4	8	1	3	4	6	1	1	2	1	1	1	61
1220	44	40	4	4	6	9	1	6	14	14	1	1	1	2	4	1	152
1221	12	13	2	2	1	1	2	4	3	1	1	1	1	1	1	1	37
1222	31	18	4	1	4	1	6	5	9	1	1	1	1	1	1	1	83
1225	21	13	2	2	1	3	1	2	4	7	1	1	1	1	1	1	59
1230	11	20	1	7	5	3	2	1	4	7	1	1	1	1	1	1	64
1231	46	33	6	4	6	2	3	13	10	1	2	1	2	1	1	1	129
1232	11	13	1	3	3	3	1	3	8	4	1	1	1	1	1	1	50
1233	27	40	4	1	4	3	6	14	11	11	1	1	1	2	1	1	127
1235	12	16	3	6	2	5	4	1	3	4	1	1	1	2	1	1	62
1236	25	19	2	7	3	1	3	11	4	1	1	1	1	1	1	1	77
1237	10	7	4	5	2	4	1	6	7	1	1	1	1	1	1	1	54
1238	12	14	2	1	2	1	3	4	1	1	1	1	1	1	1	1	41
1240	27	27	3	4	4	9	5	4	10	1	1	1	2	1	2	3	103
1241	26	27	4	3	2	2	6	6	13	1	1	1	2	1	1	1	94
1243	44	36	5	3	4	3	7	2	18	15	2	2	3	2	1	1	147
1244	12	10	2	3	1	2	2	5	5	6	1	1	1	1	1	1	48
1247	30	30	2	5	4	5	4	4	9	9	1	1	1	4	2	1	109
1253	38	30	4	5	6	4	4	7	8	11	4	2	1	1	2	1	132
1254	33	28	7	8	2	6	4	8	7	11	1	1	1	1	1	1	118
1256	33	29	8	3	7	5	3	4	6	11	1	1	1	1	1	1	111
1257	27	37	8	5	5	5	6	4	7	14	1	1	1	1	1	1	120
1258	39	26	6	4	2	2	4	3	13	15	1	1	2	4	1	2	125
1259	22	15	6	4	1	5	2	12	14	1	1	1	1	1	1	1	72
1260	26	34	3	6	3	1	9	5	9	10	1	1	2	1	1	2	112
1261	22	16	3	7	2	2	6	6	8	1	1	1	1	1	1	1	77
1262	30	38	2	2	2	8	6	7	3	13	1	1	3	1	1	1	121
1263	40	46	4	3	5	7	9	6	11	8	2	1	1	3	2	2	150
1264	14	12	2	2	1	4	2	5	14	2	1	1	1	1	1	1	59
1265	12	8	2	2	1	9	2	2	2	4	1	1	1	2	1	1	40
1266	17	11	1	3	4	4	2	2	2	2	1	2	1	1	1	3	54
1267	23	20	5	2	6	7	4	6	9	8	2	1	2	1	1	1	101
1268	22	27	5	1	4	4	1	3	10	8	1	1	1	4	2	1	96
1281	39	50	8	14	7	6	4	10	10	17	1	2	1	4	1	1	174
1282	63	42	11	10	7	11	8	14	16	9	1	1	1	3	1	1	207
1283	17	20	12	7	4	3	3	4	6	1	1	1	1	1	1	1	72

TABLE D (continued)

References	0	1	2	3	4	1,2	1,3	1,4	2,3	2,4	3,4	1,2,3	1,2,4	1,3,4	2,3,4	1,2,3,4	Total
1284	28	9	2	2	1	5	2	4	2	3	2	1	1	1	1	1	66
1287	29	25	7	3	4	4	8	5	4	10	2	2	2	1	2	1	113
1288	64	21	1	2	4	4	5	6	7	8	2	1	1	1	2	1	130
1289	30	29	5	6	5	8	7	8	6	13	2	2	1	1	1	1	123
1290	42	38	9	9	10	7	8	11	15	14	4	2	1	2	3	2	189
1291	45	48	6	10	15	13	2	7	7	6	1	2	3	3	2	1	180
1292	33	23	8	4	7	6	6	3	12	3	2	4	3	1	2	1	129
1293	41	35	7	5	9	8	9	4	11	8	3	1	1	1	1	1	150
1294	39	41	4	4	10	13	12	6	15	15	1	3	3	3	1	1	181
1296	40	43	11	4	1	9	4	6	15	16	3	1	1	4	2	2	166
1297	28	19	3	4	4	7	3	9	11	1	1	1	1	1	1	1	97
1298	47	71	6	8	6	6	11	8	11	12	1	1	4	2	2	3	199
1301	35	29	5	7	1	6	5	4	9	18	2	1	1	1	3	1	131
1302	32	24	6	2	2	5	2	2	2	11	1	1	1	1	1	1	91
1304	37	30	6	2	4	5	6	8	9	2	1	1	1	1	1	1	111
1305	33	30	6	7	3	7	9	7	6	5	4	1	2	2	1	3	131
1306	14	14	2	3	1	3	3	3	2	1	1	2	1	1	1	1	54
1307	44	41	7	7	6	9	10	9	12	14	1	1	1	2	1	1	175
1308	38	34	9	6	6	9	4	6	12	15	1	2	1	1	1	1	147
Total	17,171	2,208	2,211	2,639	5,163	2,921	3,921	6,291	13,213	4,113	1,251	11	39	17	13	1	31,456

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SEX DETERMINATION IN THE WHITE FLY

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INTRODUCTION

It is reported by WILLIAMS (1917) that in the common "white fly," *Aleurodes vaporariorum*, parthenogenetic eggs yield females among the English representatives of the species, but males in the United States. This statement is based on experiments with virgin females in both countries. On the other hand, two females of the English strain which had mated produced in the one family 13 females and 11 males, in the other family 2 females and 2 males. From these results WILLIAMS concludes that fertilized eggs of this species produce males and females in equal numbers, and applies his conclusion alike to the English and American strains. He also proceeds to explain several other well known instances of peculiar sex ratios in a similar manner, particularly among the Thysanoptera.

We have been unable to discover any other reported case of a species in which unfertilized eggs produce males while fertilized eggs yield both sexes. In such a species the males must be of two kinds, one produced from parthenogenetic eggs, the other from fertilized eggs. A species in which these two kinds of males existed would be invaluable in breeding experiments, owing to the presumably different capacities of the males for transmitting hereditary traits. For this reason we would not willingly

reject WILLIAMS's explanation of sex determination in Aleurodes, if we were not forced to do so. One of us (SHULL 1917) conducted numerous experiments on *Anthothrips verbasci* in the hope of finding that this species was of the kind that Aleurodes is reported to be, only to be driven to conclude that that thrips is precisely like the honey bee as regards sex. The case of Aleurodes seemed, therefore, to require further investigation.

The conclusion that the fertilized eggs of Aleurodes yield both sexes in equal numbers rested on the erroneous assumption that all eggs laid by mated females must be fertilized. It is well known that the female bee may and does lay unfertilized eggs while her spermatheca is full of spermatozoa; and SHULL (1917) found the same to be true of *Anthothrips*. If it be assumed that Aleurodes is like the bee and *Anthothrips* in this regard, the sex ratios obtained by WILLIAMS are quite as well explained as by his assumption that fertilized eggs may be of either sex.

To decide between these two possible explanations one might resort (1) to cytological study of the males, which on the one view should be all of one kind, and on the other view of two kinds probably differing in their chromosome number; or (2) to breeding experiments in which the males should transmit the characters of one parent or two, according as they came from parthenogenetic or fertilized eggs; or (3) to a study of the sex ratios obtained among the offspring of virgin and mated females. Believing that, if the facts should prove to be as we suspected they would, the third method entailed the least labor, we have employed the method of sex ratios. Let us see what sex ratios are permitted by the alternative theories.

(A) If Aleurodes is like the honey bee, and males come only from unfertilized eggs while all fertilized eggs produce females, the sex ratio in the family of a mated female may be anything whatever. Her offspring may be all females if every egg is fertilized, or they may be all males if in mating no spermatozoa are transferred or if the spermatozoa are withheld. Between these extremes may be any proportion of the sexes.

(B) If, as WILLIAMS believes, parthenogenetic eggs yield males, and fertilized eggs produce both sexes in equal numbers, the number of females could never exceed fifty percent. The maximum number of females would appear when all eggs were fertilized. If any developed parthenogenetically, the number of males would be increased, and the proportion of females would fall below fifty percent.

The method of sex ratios can only decide between these theories in case (A) is correct, and if in some instance a significant majority of females is obtained. If (B) is correct that fact can not be proved by sex ratios; and any proportion of females not over fifty percent fits either theory equally well. As shown below, we believe we have established such female majorities in several instances.

METHODS

The white flies¹ were reared on bean and potato plants, and a few others, under lantern globes covered with fine gauze. To prevent the plants from growing too large during the rather long life cycle of the insects, and likewise to prevent fungus from developing under the covers, watering was reduced to a minimum. Virgin females were obtained by collecting pupae and permitting the adults to emerge in confinement. In experiments in which fertilized eggs were desired, in some cases mating was observed, in other cases the females were simply confined with males and pairing may or may not have occurred. In collecting the adult offspring for ascertaining their sex, if only a few were present, a brush and a bottle sufficed and but few specimens were lost. When large numbers were to be collected advantage was taken of the positive phototropism of the flies. The plant bearing them was placed in a tight box lined with black paper on all sides except one, into which a large glass funnel was fitted with stem outward. This funnel was turned toward the window and a bottle placed over the open stem. The flies gradually collected in this bottle, in which they were subsequently etherized. The repeated use of this cage in collecting flies from different plants made errors possible, and in one or two instances we suspect the results are incorrect for this reason; but great care was exercised to remove all flies from the box, and on the whole contamination seems to have been prevented.

SEX RATIO IN RANDOM COLLECTIONS

Random collections in several greenhouses were made up of males and females in the proportions shown in table 1. Although the females are in the majority, the majority is small; and if the females live longer than the males, as WILLIAMS (1917) suggests, the sexes might have been present in equal numbers at the time of hatching. The "wild" collections

¹ The species used in our experiments is tentatively identified from adults, by Dr. A. L. QUAINANCE and Dr. A. C. BAKER, as probably *Trialeurodes* (*Aleurodes*) *vaporariorum*. Unfortunately no pupae, upon which the positive determination depends, were preserved from the experiments.

TABLE I

Proportions of the sexes of Trialeurodes vaporariorum in random collections.

Place of collection	Number of males	Number of females
University greenhouse	340	491
Botanical greenhouse	310	375
Commercial greenhouse	694	747
Total	1344	1613

do not, therefore, aid in deciding between the two theories of sex determination outlined above.

OFFSPRING OF VIRGIN FEMALES

Experiments in which virgin females were used as parents were conducted in two ways. In the first experiments the parents were left on the plants until the oldest offspring became adult, so that the parents may have appeared in the collections of progeny. In the later experiments

TABLE 2

Showing the sex ratio of the offspring of virgin females, in experiments in which the parents may be included with the offspring.

Number of experiment	Date of beginning experiment	Number of virgin parents	Offspring		
			Male	Female	Not determined
V ₁	February 20	7	138	4	4
V ₂	February 21	8	298	6	24
Total			436	10	28

the parents were removed before any of the offspring matured, so that later collections included only the progeny. The early experiments, two in number, are recorded in table 2. In neither experiment is the number of females included among the offspring greater than the number of female parents used, so that the females recorded need not be offspring at all. In further support of this view is the fact that the females obtained were among the first collections, and that no subsequent females were found.

The experiments with virgin females, in which the parents were removed before the first offspring became adult are recorded in table 3. Our experiments confirm the statement of WILLIAMS that *unfertilized eggs produce only males*.

TABLE 3

Showing the number of males and females among the offspring of virgin females, in experiments in which the parents were removed before any of the offspring became adult.

Number of experiment	Date of beginning experiment	Number of virgin parents	Offspring		
			Male	Female	Not determined
V3	February 23	3	2	0	0
V3a	March 1	3	51	0	4
V10	April 19	2	28	0	0
V20	April 29	2	64	0	0
V27	May 11	5	2	0	0
Total			147	0	4

OFFSPRING OF FEMALES THAT MATE

Mating not observed

In a series of experiments mating was permitted to take place by putting males and females under the same cover, but was not observed to occur. The results of these experiments are collected in table 4. The

TABLE 4

The offspring of females which were kept in the presence of males, but which were not observed to mate.

Number of experiment	Date of beginning experiment	Number of parents		Offspring		
		Male	Female	Male	Female	Not determined
M2	March 7	35	20	8	21	0
M3	March 7	15	9	47	39	5
M6	March 9	37	22	0	2	1
M8	March 25	31	25	37	27	1
M9	March 27	15	13	63	33	11
M10	March 30	35	34	63	53	5
M11	March 29	9	9	55	9	4
M14	March 30	30	23	351	324	8
M22	April 13	8	3	19	49	0
M25	April 17	11	3	56	76	0
M26	April 18	5	3	48	126	0
M27	April 19	4	2	86	68	0
M28	April 19	4	2	54	67	0
M36	May 10	8	2	2	0	0
Total				889	894	35

only one of these experiments which exhibits a decided majority of females, and is therefore significant in deciding between the two alterna-

tive theories of sex determination outlined in the introduction, is M26. How significant this experiment is is indicated below.

Mating observed

In the experiments shown in table 5, pairs of flies in copulation were separated, and the females alone put under lantern globes. Mating was thus known to have occurred, but could not have occurred repeatedly during the course of the experiment, as may have happened in table 4. Two of these experiments yielded a great majority of females.

TABLE 5

The offspring of females which were observed in copulation, but which were then isolated so that further mating was prevented.

Number of experiment	Date of beginning experiment	Number of parents	Offspring		
			Male	Female	Not determined
V15	April 20	1	11	1	1
V21	April 30	3	66	205	0
V26	May 9	3	9	50	9
Total			86	256	10

The production of a large majority of females in the experiments of table 5 is probably owing to the fact that the parents had certainly mated before the beginning of the experiment. It suggests that in the experiments of table 4 some of the females did not mate at first, or may even not have mated at all, and that the small proportion of females was due to the laying of unfertilized eggs by such virgin females, rather than to the withholding of spermatozoa by females that had mated.

SIGNIFICANCE OF THE EXPERIMENTS WITH MATED FEMALES

Three of the experiments with females that were either known to have mated or were given every opportunity to mate resulted in so large a majority of females as to appear to be decisive with regard to the method of sex determination. These are M26, V21, and V26. The fact that there were only three such experiments, whereas many others intended to be like them yielded only an equality or even a minority of females, does not diminish their value as evidence. Even if only one experiment in a thousand showed a majority of females, and that majority was sufficiently large, that one experiment would decide between the alternative views described in the introduction. It is important, therefore, to ascertain how significant the female majorities in these three cases are.

It may be objected that even if the two sexes were regularly produced in equal numbers, a random sample could, purely by chance, be composed of three to five times as many females as males. It may be argued that, even if there are in the spermatheca of a female fly two kinds of spermatozoa, respectively male- and female-producing, in equal numbers, the eggs laid may, purely by chance, be fertilized mainly by spermatozoa of the female-producing type. These chances do exist. How great they are can be computed.

Let us examine the totals of the three experiments, M26, V21, and V26. Assume that the flies recorded in tables 4 and 5 were drawn at random from an infinitely numerous population composed of equal numbers of males and females. The chance (probability) that any given sex ratio would occur in a sample is expressed by the formula

$$\frac{n!}{(n-r)!r!} p^r q^{(n-r)}$$

in which n is the total number of individuals in the sample, r is the number of females in such sample, p is the chance that any selected individual will be a female, q the chance that any selected individual will be a male. Under the conditions named above, $p = q = \frac{1}{2}$, while n and r vary with the samples.

Employing this formula, it is found that the chance that the 48 males and 126 females in experiment M26 might have come from a population in which males and females were equally abundant is

$$\frac{174!}{48! \times 126!} \left(\frac{1}{2}\right)^{126} \left(\frac{1}{2}\right)^{48} = \frac{174!}{48! \times 126! \times 2^{174}}$$

With the slight error due to the use of logarithms this chance proves to be 1 in 1,097,307,692. In like manner the chance that the ratio in experiment V21 could be 66 males and 205 females, and still represent an infinite population in which the sexes were equally numerous, is 1 in 310,942,857,142,857,142. The corresponding probability for experiment V26 is 1 in 45,876,666.

These figures, of course, only express the chance that the one specific ratio should occur. If one were to compute the chance that the ratio might be at least as far from equality as is the ratio actually obtained, it would be necessary to combine with the chance of the one ratio obtained the chances of all other ratios still farther from equality. Thus, in experiment V21, to the probability of the ratio 66 to 205 should be added

the probabilities of the ratios 65 to 206, 64 to 207, 63 to 208 . . . 1 to 270, 0 to 271. Since all these latter chances are less than that of the ratio 66 to 205, they may be combined and their sum still be only 1 in some quadrillions.

Contrast with these figures the chance that, in the same experiment (V21), the ratio might have been as near equality as possible, that is, 135 to 136. That chance is 1 in 20.6. Out of a large number of samples, of 271 individuals each, all drawn as hypothecated from an infinite population in which males and females are equally numerous, more than half of them should deviate less than 6 from equality. The number falling beyond the extreme ratios of 100 to 171, and 171 to 100, would be negligible.

It should be pointed out that the assumption of an infinite population from which to select the given samples is less favorable to the conclusion we propose to draw from the experiments than any finite population

TABLE 6
Daily records of the progeny obtained in experiments M26, V21, and V26.

Experi- ment	Date of collection	Number of males	Number of females	Percentage of males
M26	May 19	3	18	14.3
	May 30	11	32	25.6
		18	18	50.0
		7	11	38.9
		1	3	25.0
	May 31	0	4	0.0
		1	11	8.3
	June 4	3	14	17.6
	June 13	4	15	21.1
		48	126	27.6
V21	May 30	7	58	10.8
		6	15	28.6
		0	6	0.0
	June 17	26	62	29.5
		24	51	32.0
		2	11	15.4
	June 20	1	2	33.3
		66	205	24.4
V26	June 17	1	15	6.2
	June 29	2	32	5.9
	July 4	6	3	66.7
		9	50	15.3

would be. If samples were drawn at random from any finite population, in which the sexes were equally abundant, the probability of any given one-sided ratio, such as 66 to 205, would be less than that computed above.

The above computations are based solely upon the total sex ratio obtained. They take no account of the nature of the individual collections within the experiments. These, however are important, and are given in table 6. It is noteworthy that with two exceptions each collection contained a majority of females. The fact that they are nearly all of the same sign enhances the probability that they represent a population in which the females are more numerous than the males.

The practical uniformity of sign of the several collections could be given expression and its significance shown by treating all the samples in each experiment by statistical methods. In view of the small number of collections in each experiment, however, the value of such treatment seems doubtful; and inasmuch as the computations already made appear wholly conclusive, we have dispensed with further refinements.

Experiment V26 may appear from table 6 to be less satisfactory as proof that the true proportion of males is less than 50 percent than are the other two experiments. But when it is observed (1) that most of the apparent weakness of this experiment lies in its last collection in which the males are in the majority, (2) that the females which produced these offspring mated only before and not during the experiment, and (3) that their supply of stored spermatozoa may have been exhausted by the fertilization of the earlier eggs, this final majority of males may well be regarded as a confirmation of the theory of sex determination which the preceding female majorities seem to render necessary, namely, that fertilized eggs yield females, unfertilized eggs males.

DISCUSSION AND SUMMARY

The experiments described above indicate with little doubt that unfertilized eggs of *Trialeurodes vaporariorum* produce males, which confirms the statement of WILLIAMS with regard to the American representatives of this species. They should dispel, however, the belief that the fertilized eggs produce both sexes in equal numbers. If that belief were well founded, it should never be possible to obtain a significant majority of females, for to the fifty percent of males developed from fertilized eggs must be added the males which hatch from parthenogenetic eggs. In three experiments a very large majority of females was obtained. This result favors the view that, like the eggs of the

honey bee, all fertilized eggs of the white fly produce females, all parthenogenetic eggs males, in accordance with which view the sex ratios may be anything whatever. The only alternative explanations of the majorities of females in the three experiments above referred to would be selective fertilization and differential mortality. Since there is no indication that either of these phenomena occurs, and since analogy with the honey bee is at least probable, we have adopted the view that all fertilized eggs yield females.

While our conclusion is what we suspected at the outset that it would be, we believe that our judgment has been in nowise distorted by our preconception. We are, in fact, very desirous of discovering just such an animal as WILLIAMS describes this *Trialeurodes* to be. It is to be hoped that any indications that any species produces males from unfertilized eggs, and both sexes from fertilized eggs, will be promptly and thoroughly investigated.

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INHERITANCE OF SPOTTED ALEURONE COLOR IN HYBRIDS OF CHINESE MAIZE¹

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The color in the aleurone cells of maize early attracted the attention of geneticists as affording ideal material for testing the segregation of characters in the second generation of hybrids.

The first cases that were investigated led to the idea of only one unit or factor being involved in the production of an aleurone color. With the progress of investigation, however, it became necessary to assume that two factors are involved. The occurrence of red and purple seeds on the same ear showed the need of recognizing a third factor, and the appearance of a pale color, or "dilute," necessitated the assumption of a fourth, modifying, factor.

A brief examination of the many varieties grown by the Indians of North and South America shows that there are still other aleurone colors not provided for in this scheme, such as clay (a light blue but very distinct from dilute) found in varieties grown by the Hopi Indians of Arizona; pink (a light red), isolated from the Chinese waxy type, and black (an exceedingly intense blue or purple) found in varieties grown by the Navajo Indians and brown found in a Bolivian variety. It is not absolutely certain as yet whether additional factors are concerned in the production of these variations, or whether we are dealing with one or more dilution factors similar to those found in guinea pigs (WRIGHT 1916).

Apart from the many shades of color there are several color patterns corresponding in a certain sense to those of animals. Not all of these patterns have been, as yet, thoroughly investigated, but they are so distinct in appearance that there can be little doubt that additional factors or heritable elements are involved in their expression.

While at least two color schemes found on maize seeds are undoubtedly

¹ Since this manuscript was prepared a paper by Professor R. A. EMERSON (1918) has appeared, in which a similar type of aleurone color has been analyzed and designated mottled. In general the conclusions reached are similar to those presented here, but there are some differences which it is believed will be of interest.

patterns, two others would be classed more properly perhaps as a color dilution. If this is true there are two types of color dilution in the aleurone layer. The first of these has color in each aleurone cell but of greatly reduced intensity. This type is found in both red and blue. Most of the seeds belonging to it are darker at the base, the color paling gradually toward the tip. This type may possibly correspond to that designated parti-colored by EAST and HAYS (1911).

The other type of color dilution found in maize is perhaps comparable to the blue roan color in horses and cattle, in that with respect to color, there are two kinds of cells, those with pigment and those without. Unlike the blue roans, where cells of a color do not occur in groups, the seeds of maize are flecked with spots of irregular size, scattered indiscriminately over the entire surface and seemingly showing no evidence of concentration either at the base or the tip.

Cells of a color occur in groups of varying sizes. In many cases the color, however, is intense, the seed appearing lighter in shade only when groups of colored cells alternating with groups of colorless cells are extremely small. Cases occur in which the two types of dilution are combined, the seeds being spotted with a faint color.

Chemically it is quite possible that the two types of color dilution found in the aleurone layer of maize seeds represent two conditions of a single enzyme which causes the development of anthocyanin through the oxidation of a chromogen, a suggestion already advanced by WRIGHT (1917) to account for a parallel case in the melanin pigment of mammals.

Where the intensity of the pigment itself is reduced, the entire seed being but faintly colored, it may be that the enzyme is weak and slow to react.

While in the spotted type of dilution in which the color is intense but does not extend over the entire seed, it would appear that the enzyme is sufficiently active, but the quantity may be reduced to such an extent that enough chromogen can not be oxidized to cover the entire seed.

The occurrence of spotted seeds in a cross between the Chinese white waxy corn and a colored Algerian pop corn has given the opportunity to study the inheritance of this spotted type of color distribution.

There are several peculiarities in the production of spotting which are not inharmonious with the supposition that this type of color distribution is in reality a color dilution, due perhaps to a reduced quantity of an enzyme concerned in the production of color.

The factors which influence the appearance and production of color

in the aleurone cells of maize, so far as investigated and reported, are listed below:

C, a factor for the production of any color, but inactive unless another factor (which has been designated *R*) is present.

R, a factor as necessary as *C* for the production of color. With *C* it will produce a red color. It may be distinguished from *C* because the latter factor is correlated with the texture of the endosperm.

A, a factor reported by Emerson (1918) as necessary for the production of any color.

P, a factor that in combination with the factors *C* and *R* changes the color of the aleurone from red to blue or purple.

I, a factor concerned with the inhibition of color. It is assumed to affect the production of both red and blue.

S. This factor is here reported for the first time. It is concerned with the production of a certain type of minute spotting and seems to be associated or correlated with the factor *R*.

In 1910 crosses were made between a variety of maize received from Algeria, having colored aleurone and horny endosperm and a Chinese variety with white waxy endosperm. One cross was made with the Algerian variety as the male parent, the other with this variety as the female parent. Both of the resulting ears were uniform or self-colored, in this respect resembling the Algerian parent. The progeny of the ear having the Algerian plant as the male parent failed to show the usual association of endosperm texture and aleurone color.

This result seemed to afford ground for the belief that there might be a difference between reciprocal crosses with respect to the association of aleurone color and endosperm texture. To investigate this possibility, six crosses were made between these same two varieties in 1913. While no differences in the association of the characters were found between reciprocal crosses, the results showed conclusively that in these hybrids aleurone color may show different distributions in the seeds of reciprocal crosses.

Of the six hybrid ears three were borne on plants of the Algerian variety, the other three on plants of the Chinese variety. The former were self-colored and could not be distinguished from pure Algerian ears. The latter were also colored, but the color on two of these was in numerous small spots. Of these two, one had all the seeds spotted, the other approximately half of the seeds spotted, the actual ratio being 107 spotted to 97 self-colored. The relationship of the six ears is shown in figure 1.

It is to be noted that the Algerian plants, which bore the self-colored

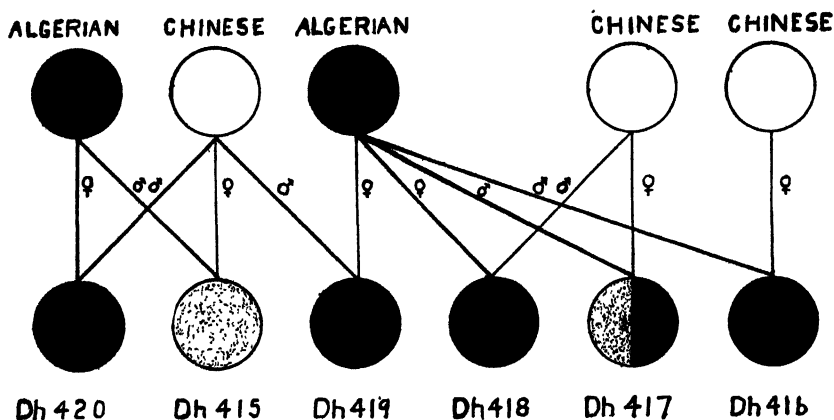


FIGURE 1.—Diagram showing the relationship of the 6 hybrid ears.

ears Dh420 and Dh419, showed an incomplete dominance when used as male parents with some Chinese plants, but a complete dominance when these same Chinese plants were used as male parents. That the dominance of the Algerian color is not always incomplete when Algerian plants serve as male parents is demonstrated by the hybrid ear Dh416 which had self-colored kernels.

Plants from all six ears were grown in 1914, and a large number of hand-pollinated ears bearing seeds representing the second generation were secured. The progeny of all but one of the six ears produced ears with some spotted seeds. The one exception, ear Dh416, was borne on a Chinese plant and was pollinated from the same Algerian plant that served as the male parent of ear Dh417, which was spotted.

The progeny of the remaining 5 ears produced 60 hand-pollinated ears having some of the seeds spotted. Of these 35 showed a dihybrid ratio of white to colored seeds, and 2 had all the seeds colored. It must be borne in mind, however, that these 60 ears were the result of self-pollination, and crosses between plants grown from the other hybrid ears.

As both red and blue seeds were found on ears having a dihybrid ratio of white to colored seeds, it was necessary to assume that three factors are involved in the production of color; (1) A basic factor (C), which is essential for the production of any color, (2) a factor for red (R) which in conjunction with the factor C results in a red seed; and (3) a factor for blue (P_r), which, when present in the same zygote with the factors C and R , results in these seeds being blue. All com-

binations of these factors other than C and R , or C , R , and P_r , result in the production of white seeds. The suggestion has been advanced that the factor correlated with endosperm texture is the basic factor C (KEMPTON 1915, BREGGER 1918). This is mentioned here, as it affords a method of distinguishing between the factors C and R , which is of

TABLE I
Percentage of spotted seeds on ears with a dihybrid ratio of white to colored seeds.

Pedigree number	Total number	Number colored	Number spotted	Percentages of total spotted	Percentage of colored that are spotted	Percentage of white
2250	86	52	5	5.8	9.6	39.5
2261	648	369	104	16.1	28.2	43.0
2262	438	252	53	12.1	21.1	42.4
2263	251	153	36	14.3	23.5	39.0
2264	517	278	75	14.5	27.0	46.4
2265	345	199	49	14.2	24.6	42.3
2266	606	332	83	13.7	25.0	45.2
2301	218	124	35	16.1	28.2	43.2
2302	435	259	86	19.8	33.2	40.4
2311	178	110	28	15.7	25.4	38.2
2312	244	131	31	12.7	23.7	46.4
2315	508	288	100	19.7	34.7	43.3
2316	480	254	85	17.7	33.4	47.1
2324	283	168	61	21.5	36.3	40.6
2325	159	89	30	18.9	33.7	44.0
2326	309	177	55	17.8	31.1	42.7
2331	294	174	46	15.6	26.4	40.8
2332	526	286	104	19.8	36.4	45.6
2336	392	221	72	18.3	32.6	43.6
2339	595	358	129	21.7	36.1	39.8
2340	537	285	97	18.1	34.0	47.0
2342	357	208	70	19.6	33.6	41.7
2343	265	139	43	16.2	30.9	47.5
2347	395	226	80	20.3	35.4	42.8
2348	44	26	5	11.4	19.2	41.0
2350	311	165	64	20.6	38.8	47.0
2351	52	29	10	19.2	34.5	44.3
2352	565	320	89	15.8	27.8	43.4
2353	230	135	73	31.7	54.1	41.3
2356	454	268	80	17.6	29.8	41.0
2358	251	148	44	17.5	29.7	41.0
2359	512	309	105	20.5	34.0	39.6
2364	526	286	75	14.3	26.2	45.6
2365	34	13	3	8.8	23.1	61.7
2366	407	252	89	21.8	35.3	38.1
Total	12452	7083	2194	17.6	30.4	43.2

importance in analyzing the inheritance of spotted seeds, as will appear later.

All the ears with a dihybrid ratio of white to colored seeds had some seeds that were spotted. The percentage of the total seeds that showed spotting was $17.6 \pm .236$, but since spotting can be detected only when the seeds are colored, it would seem that the percentage of spotted seeds on an ear should be determined by the percentage of colored seeds that are spotted. If this assumption is admitted the percentage of spotted seeds becomes $30.4 \pm .277$ for these hybrid ears.

The ears having a monohybrid ratio of white to colored seeds fell into two groups as regards spotted seeds—those having some of the seeds spotted and those without any spotted seeds.

Disregarding for the present those ears without spotted seeds, the percentage of spotted seeds on the others is $21.7 \pm .27$, but the per-

TABLE 2

Percentage of spotted seeds on ears with a monohybrid ratio of white to colored seeds.

Pedigree number	Total number	Number colored	Number spotted	Percentages of total spotted	Percentage of colored that are spotted	Percentage of white
2251	268	204	63	23.5	30.8	23.9
2252	39	29	8	20.5	27.6	25.6
2256	627	484	92	14.7	19.0	22.8
2257	574	436	138	24.0	31.7	24.0
2259	576	421	103	17.9	24.5	26.9
2260	518	397	87	16.8	21.9	23.4
2303	267	185	44	16.5	23.8	30.7
2308	600	440	145	24.2	33.0	26.6
2319	402	279	111	27.6	39.8	30.6
2333	579	403	155	26.8	38.5	30.4
2334	260	193	70	26.9	36.2	25.8
2335	399	293	93	23.3	31.7	26.5
2337	497	386	132	26.6	34.2	22.3
2338	400	292	83	20.8	28.4	27.0
2344	564	409	129	22.9	31.5	27.5
2346	588	452	145	24.7	32.1	23.1
2349	605	432	112	18.5	25.9	28.6
2355	202	156	48	23.7	30.8	22.7
2357	288	221	49	17.0	22.2	23.3
2360	618	466	142	23.0	30.5	24.6
2362	554	408	129	23.3	31.6	26.4
2363	225	161	52	23.1	32.3	28.4
2345	471	313	66	14.0	21.1	33.5
Total	10121	7460	2196	21.7	29.4	26.3

centage of colored seeds that are spotted is $29.4 \pm .30$, essentially the same percentage as was observed on the ears with a dihybrid ratio of white to colored seeds (see table 2). The percentage of spotted seeds based on the total can not, of course, be alike on ears with monohybrid and dihybrid ratios of white to colored seeds, since the latter class has a relatively larger percentage of the total seeds white.

A plant of the hybrid Dh420 when crossed with a plant of the hybrid Dh417 resulted in the production of an all-colored ear. A similar result was secured when a plant of the hybrid Dh415 was crossed with another plant of the hybrid Dh417. The assumption in these cases is that one of the plants is genotypically constituted *CCRr* and the other *CcRR*. These two all-colored ears had $29.1 \pm .82$ of the seeds spotted (table 3). They are represented diagrammatically in figures 2 and 3 (pp. 272, 273).

Thus it is seen that ears with a dihybrid and those with a hybrid ratio of white to colored seeds, as well as ears having all of the seeds colored, have essentially the same percentage of the colored seeds spotted, provided, of course, that they have spotted seeds at all.

Combining the three classes of ears, those with a monohybrid and dihybrid ratio of white to colored seeds as well as the two ears having all the seeds colored, the percentage of colored seeds that are spotted is found to be $29.9 \pm .19$. This percentage at first glance might be considered an approximation of the 25 percent expected for a simple monohybrid ratio, but the deviation is 23.8 times the probable error.

TABLE 3
Percentage of spotted seeds on ears having all the seeds colored.

Pedigree number	Total number	Number colored	Number spotted	Percentages of total spotted	Percentage of colored that are spotted	Percentage of white
2254	699	699	191	27.3	27.3	0
2361	608	608	189	31.1	31.1	0
Total	1307	1307	380	29.1	29.1	0

As all of the ears having a dihybrid ratio of white to colored seeds had some of the seeds spotted and, further, as approximately one-half of the ears with a monohybrid ratio of white to colored seeds (the progeny of the hybrid Dh416 omitted) had some of the seeds spotted, it would appear that when a plant is heterozygous for one of the factors for aleurone color some of the seeds are spotted.

Confining the analysis to those ears which were the result of self-pollination it is found that when there is no correlation between aleurone color and endosperm texture all the ears have some spotted seeds, and conversely (if two ears having only slightly more than one percent of the seeds spotted are disregarded) when there is a correlation, none of the ears has spotted seeds. The distribution is then as follows:

	Some seeds spotted	No seeds spotted
Aleurone color and endosperm texture correlated.....	0	8
Aleurone color and endosperm texture not correlated.....	7	0

The factor for color correlated with endosperm texture has been designated *C*; therefore, when an ear is secured with a correlation between aleurone color and endosperm texture, the parents must have been heterozygous for the factor *C*, and if the ratio of colored to white seeds approximates 3 to 1, the parent must also have been homozygous for the factor *R*.

The above four-fold distribution establishes the fact that in the presence of a spotting factor, *S*, spotted seeds occur only when the factor uncorrelated with endosperm texture, *R*, is heterozygous. Since in these ears the factor *C* is homozygous, it remains to ascertain whether it is the homozygous *C* or the heterozygous *R* that determines the occurrence of spotting. This question is answered by the fact that spotted seeds are always found on ears with a dihybrid ratio of white to colored seeds. Since the parents of such ears were heterozygous for *C*, it follows that spotting is not dependent on *C* being heterozygous, and the alternative must be accepted, that spotting occurs only when the factor *R* is heterozygous.

A further refinement of this statement is necessary to account for spotted seeds on the two ears which were the result of crossing two plants, one genotypically constituted *CCRr*, the other *CcRR*. These ears were of course all colored but had 29.1 percent of the seeds spotted (table 2).

It is found that when the self-pollinated female parent produces some of the colored seeds spotted, and the male parent all the colored seeds self-colored, crossing these two will result in the production of an ear having some of the colored seeds spotted; but if this parentage is reversed

the female parent, when self-pollinated, producing all of the colored seeds self-colored, and the male parent producing some of the colored seeds spotted, a cross between them will have all the colored seeds self-colored. This harmonizes with the assumption that spotting can be detected only when the maternal parent is heterozygous for the factor *R* or homozygous for its allelomorph *r*. On this assumption the Chinese parent of the hybrids Dh417 and Dh418 must have been heterozygous for the factor *R*, and since both monohybrid and dihybrid ears were produced, all having correlations between aleurone color and endosperm texture, the genotypic composition must have been *ccRrss*, and the genotypic composition of the Algerian parent of these hybrids must have been *CCRRSS*.

The Chinese parent of Dh415 and Dh420 must have had the genotypic composition *Ccrrss*, the Algerian parent having the composition *CCRRSS*, since the progeny of these reciprocals had both monohybrid and dihybrid ratios, but the ears with a monohybrid ratio of white to colored had no correlation between aleurone color and endosperm texture. These ears were, therefore, homozygous for the factor *C*.

Since the hybrid Dh416 was not spotted and its progeny all had a monohybrid ratio of white to colored seeds, and since all of these ears had a correlation between aleurone color and endosperm texture, the Chinese parent must have been genotypically constituted *ccRRss*, and the Algerian parent must have been constituted *CCRRSS*, similar to the other Algerian plants. The progeny of this cross are all homozygous for the factor *R*; no spotted are to be expected and none was observed.

The evidence therefore appears complete that spotted seeds occur only when the female parent is heterozygous for the factor uncorrelated with endosperm texture, this factor being designated *R*.

It is obvious from the genotypic composition necessary for the appearance of this type of spotted seeds that a strain having all of the colored seeds spotted can not be obtained, although it is possible to secure strains that will always have some of the colored seeds spotted.

Thus, spotting can be observed when the female gamete is of the class *CrS*, and the male gamete *cRS*, *CRS*, *cRs*, or *CRs*, or the female gamete of the class *Crs* and the male gamete either *cRS* or *CRS*, or the female gamete *crS* and the male gamete *CRs* or the female gamete *crs* and the male gamete *CRS*. A plant which will produce these gametic classes must be heterozygous for the factor *R*, and since this is true some of the female gametes will be of the class *CRS*. And though the zygote will be colored regardless of the composition of the male gamete, it will not be spotted.

Since in this type of spotting the zygotes are heterozygous for the factor R , no ears bearing all the seeds colored should be obtained from self-pollinating plants grown from spotted seeds, while if the spotted seeds were not heterozygous for the factor R , self-pollinating plants grown from spotted seeds, secured from ears with a ratio of 3 colored to 1 white, should give one third of the ears with all the seeds colored. At the present time 90 plants have been grown from spotted seeds of these hybrids, but none of the ears from these plants has had all of the colored seeds spotted or all of the seeds colored.

As a further test several plants grown from spotted seeds were crossed with a strain called " R tester" secured from R. A. EMERSON and all proved to be heterozygous for R . It is known that varieties of maize having all of the seeds minutely spotted are grown by the North American Indians. These varieties breed true for spotting, and it is hoped that by the end of another season it will be possible to determine the relationship of this type of spotting to that represented by the hybrids under discussion.

ASSOCIATION OF SPOTTING AND ALEURONE FACTORS

Having satisfied ourselves that spotting appears only when the female parent is heterozygous for the factor for aleurone color, R , attention may be turned to the rather unusual percentage of spotted seeds. The percentage of colored seeds that are spotted prevents any very simple explanation on the basis of multiple factors.

A satisfactory approximation of the observed percentage may be obtained by assuming a coupling or linkage between the factor for aleurone color, R , and a dominant spotting factor, S .

The best fit is secured by assuming the gametic ratio to be $7:1:1:7$, the combination RS and rs being 7 times as numerous in the gametes as the combinations rS and Rs . This ratio may be the result of the two factors being located on the same chromosome and separated by 12.5 units, or the cells bearing the associated factors may divide or reduplicate 7 times as fast as the cells bearing the odd combinations.

On this assumption a plant which, when self-pollinated, produces an ear with a monohybrid ratio of white to colored seeds, with no correlation between aleurone color and endosperm texture, and having some spotted seeds, would be making the gametic classes shown in diagram 1.

Such a series will give 135 self-colored to 57 spotted seeds or 29.68 percent of the colored seeds spotted, which is a very close approximation to the observed percentage $29.4 \pm .30$.

	7 CRS	1 CRs	1 CrS	7 Crs
7 CRS	Self	Self	Self	Self
1 CRs	Self	Self	Self	Self
1 CrS	Spot	Spot	White	White
7 Crs	Spot	Self	White	White

DIAGRAM 1.

Continuing with the assumption that the factor for color, R , and the factor for spotting, S , are correlated in the gametes in a ratio of 7:1:1:7, self-pollinating a plant heterozygous for both color factors, C and R , as well as the spotting factor, S , would give an ear with the following zygotic classes (Diagram 2):

	7 CRS	1 CRs	1 CrS	7 Crs	7 cRS	1 cRs	1 crS	7 crs
7 CRS	$\frac{49}{7}$ Self	$\frac{7}{1}$ Self	$\frac{7}{1}$ Self	$\frac{49}{7}$ Self	$\frac{49}{7}$ Self	$\frac{7}{1}$ Self	$\frac{7}{1}$ Self	$\frac{49}{7}$ Self
1 CRs	$\frac{7}{1}$ Self	$\frac{1}{1}$ Self	$\frac{1}{1}$ Self	$\frac{7}{1}$ Self	$\frac{7}{1}$ Self	$\frac{1}{1}$ Self	$\frac{1}{1}$ Self	$\frac{7}{1}$ Self
1 CrS	$\frac{7}{1}$ Spot	$\frac{1}{1}$ Spot	$\frac{1}{1}$ White	$\frac{7}{1}$ White	$\frac{7}{1}$ Spot	$\frac{1}{1}$ Spot	$\frac{1}{1}$ White	$\frac{7}{1}$ White
7 Crs	$\frac{49}{7}$ Spot	$\frac{7}{1}$ Self	$\frac{7}{1}$ White	$\frac{49}{7}$ White	$\frac{49}{7}$ Spot	$\frac{7}{1}$ Self	$\frac{7}{1}$ White	$\frac{49}{7}$ White
7 cRS	$\frac{49}{7}$ Self	$\frac{7}{1}$ Self	$\frac{7}{1}$ Self	$\frac{49}{7}$ Self	$\frac{49}{7}$ White	$\frac{7}{1}$ White	$\frac{7}{1}$ White	$\frac{49}{7}$ White
1 cRs	$\frac{7}{1}$ Self	$\frac{1}{1}$ Self	$\frac{1}{1}$ Self	$\frac{7}{1}$ Self	$\frac{7}{1}$ White	$\frac{1}{1}$ White	$\frac{1}{1}$ White	$\frac{7}{1}$ White
1 crS	$\frac{7}{1}$ Spot	$\frac{1}{1}$ Spot	$\frac{1}{1}$ White	$\frac{7}{1}$ White	$\frac{7}{1}$ White	$\frac{1}{1}$ White	$\frac{1}{1}$ White	$\frac{7}{1}$ White
7 crs	$\frac{49}{7}$ Spot	$\frac{7}{1}$ Self	$\frac{7}{1}$ White	$\frac{49}{7}$ White	$\frac{49}{7}$ White	$\frac{7}{1}$ White	$\frac{7}{1}$ White	$\frac{49}{7}$ White

DIAGRAM 2.

The ratio of self-colored to spotted seeds is 405 to 171, the same ratio as in the monohybrid ears and in accord with the observed ratios. From this evidence it would not seem unreasonable to assume that the spotting

on these seeds is due to a dominant partial-inhibiting factor which is linked or coupled with the factor for color, *R*, in a ratio closely approximating 7:1:1:7.

For the two ears with all seeds colored obtained by crossing two first-generation plants, both of which when self-pollinated produce ears with a monohybrid ratio of white to colored seeds, the percentage of spotted seeds should be reduced from 29.68 to 28.1.

These two ears are represented in figures 2 and 3.

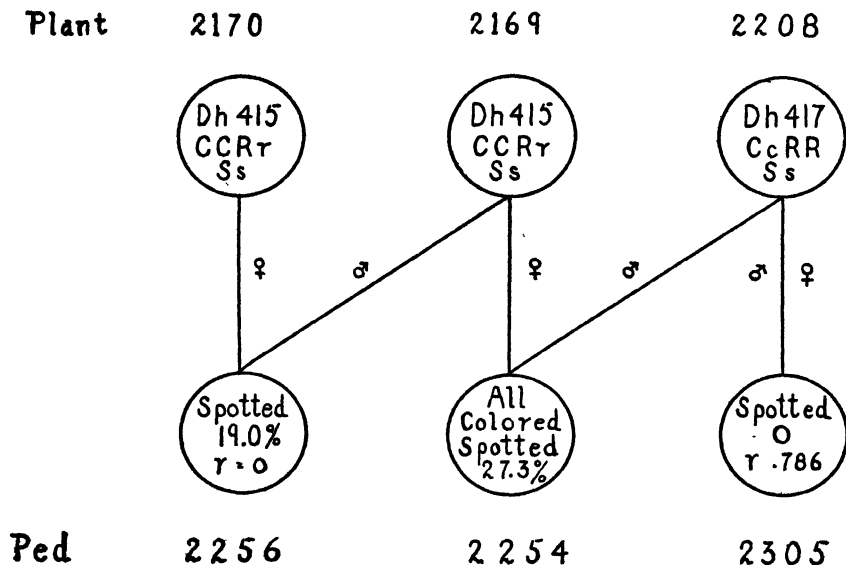


FIGURE 2.—Showing the relationship of ear 2254 which had all the seeds colored. The female parent of this ear when used as the male parent of 2256 produced 19.3 percent of the colored seeds spotted and produced no spotted seeds when used as the male parent of ear number 2305. The coefficients of association indicated on the ears 2256 and 2305 refer to the correlation between endosperm texture and aleurone color.

It is seen from this diagram that the male parent was making gametes *CRS*, *CRs*, *cRS*, *cRs*, and the female parent *CRS*, *CRs*, *CrS*, *Crs*. It is observed that no reduplication can occur in the gametes of the male parent between the factors *R* and *S*, as all of the gametes possess the factor *R*. In the female gametes, however, a 7:1:1:7 reduplication can be obtained. The gametic composition of the parents and the resulting zygotic classes are shown in diagram 3.

Were it not for the peculiar fact that spotting can appear only when the female gametes do not possess the factor *R*, the result of the gametic reduplication in the female gametes would not be apparent in the zygotes, and 37.5 percent of the seeds would be expected to be spotted.

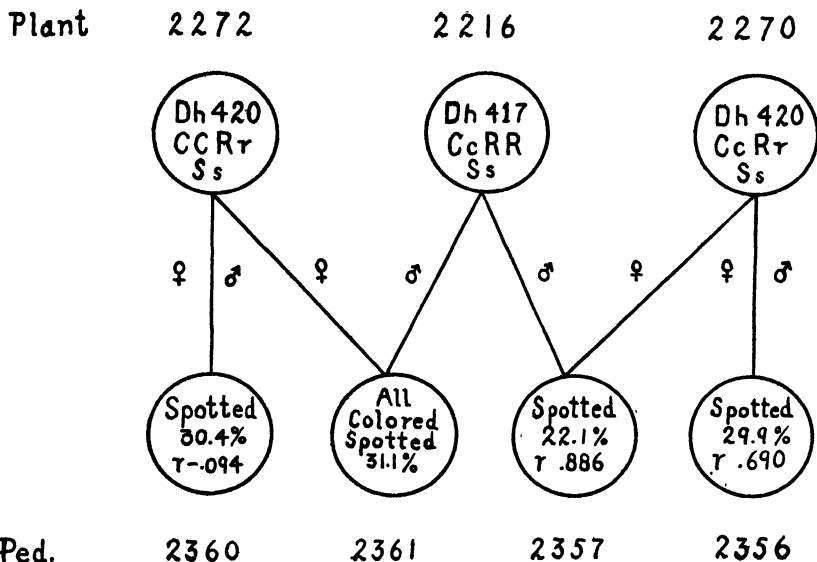


FIGURE 3.—Showing the relationship of ear 2361, an all-colored ear, with 31.1 percent of the seeds spotted. This ear was borne on the same plant, which when self-pollinated produced ear 2360. This ear (2360) had approximately 25 percent of the seeds white. The coefficients of association indicated on the ears 2360, 2357 and 2356 refer to the correlation between endosperm texture and aleurone color.

♀	♂ <i>CRS</i>	<i>CRs</i>	<i>cRS</i>	<i>cRs</i>
7 <i>CRS</i>	7 Self	7 Self	7 Self	7 Self
1 <i>CRs</i>	1 Self	1 Self	1 Self	1 Self
1 <i>cRS</i>	1 Spot	1 Spot	1 Spot	1 Spot
7 <i>cRs</i>	7 Spot	7 Self	7 Spot	7 Self

DIAGRAM 3.

The present assumption, however, gives a ratio of 46 self-colored to 18 spotted, or 28.1 percent spotted. The observed percentage for the two ears was $29.1 \pm .82$. The numbers are not sufficient to discriminate between the percentages 29.1 and 28.1, but the facts do not violate the assumption and the observed percentage of spotted seeds is actually lower in the ears with all the seeds colored.

CONCLUSIONS

Reciprocal crosses between maize plants having white and colored seeds sometimes differ in the distribution of the aleurone color.

The seeds of some of the ears borne on white-seeded plants, if pollinated from colored plants, have the aleurone color distributed in numerous small spots, while the reciprocal crosses are self-colored.

The spotting is assumed to be due to a dominant spotting factor, *S*, which is in the nature of a partial inhibitor.

From the results of this investigation it would seem that the factor which causes spotting can operate only when the factor *R* is heterozygous and furthermore is not present in the female gamete but is introduced into the zygote by the male gamete.

As the male gamete contributes but one nucleus while the female gamete contributes two, the difference between reciprocals may be ascribed to the fact that the female parent contributes twice as much chromatin material as the male parent.

It would appear, therefore, that a single nucleus does not contain enough of the factor *R* to produce a self-colored seed in the presence of the dilution factor *S*.

The percentage of spotted seeds and the fact that spotted seeds are found only on those self-pollinated ears which have no correlation between aleurone color and endosperm texture, has led to the conclusion that there is a coupling or linkage between the factor for aleurone color, *R*, and the factor for spotting, *S*.

It is to be noted further that the gametic ratio in these hybrids is an approximation to a 7:1:1:7 series, equivalent to the location of the two factors on the same chromosome, 12.5 units apart.

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CHARACTER CHANGES CAUSED BY MUTATION OF AN ENTIRE REGION OF A CHROMOSOME IN *DROSOPHILA*.¹

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A female, just hatched, having wings serrated or notched at the end, was found in the purple stock bottle, October 7, 1918. In order to test whether this character was due to a new mutation the female was mated to males from an unrelated stock. The majority of her few F_1 daughters had typically "notch" wings quite like the females in the old stocks called notch (see MORGAN and BRIDGES 1916). Later crosses in which larger numbers were obtained always gave notch females, normal females, and normal males in equal numbers, but no notch males, showing that the mutant was a sex-linked dominant, acting in addition as a lethal in the male, precisely like the old notch. It was possible to ascertain that the purple stock had not been contaminated. The character was accordingly considered as due to a reappearance of the old notch gene. Notch was known to have reappeared six times since it first occurred.

In order to find out whether the location of the new notch ("notch₈") gene was identical with that of the earlier ones, two cultures were raised in which a red-eyed notch₈ female was crossed to males carrying the sex-linked recessive genes eosin (eye color) and crimson (eye color). These genes are located fairly near to the left and to the right respectively of the old notch gene.

When the F_1 flies hatched, a paradoxical result was obtained: All the notch₈ daughters, instead of being red-eyed as expected, had a yellowish eosin-like eye color. Since the normal-winged daughters did not show this exceptional eye color, the latter could not be due to a duplication of the eosin gene in the X chromosome received from the eosin crimson fathers, and it was at once regarded as probable that the appearance of F_1 eosin notch females was due to a "deficiency" (BRIDGES 1917) in the

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X chromosome containing the notch₈ gene. If a deficiency, i.e., loss or inactivation, of a piece of the X chromosome carrying the normal allelomorph for eosin were present in the notch₈ flies, notch₈ females heterozygous for eosin would be somatically eosin, or lighter, with regard to eye color.

An important support for the deficiency-explanation was found in the fact that males which received the notch₈ chromosome all died. BRIDGES (1917) had shown that bar-deficiency acted as a lethal for the male. Moreover, notch females from two of the earlier notch stocks, in crosses to males carrying the recessive sex-linked gene for facet (eyes), located near to the left of notch, had given notch-facet females in F₁ (METZ and BRIDGES 1917).

When it was taken into consideration that notch₈ which had a peculiar effect on eosin analogous to that previously obtained with facet, also agreed with old notches in a series of somatic respects, as will be pointed out later, it was regarded as probable that the character notch itself might be due to deficiency and not to a gene of the common kind. If this were true it was to be expected that the deficiency in the notch₈ flies covered a region extending at least from eosin (at 1.1) to the locus of the old notch (at 2.6), a distance of 1.5 units. This was an extraordinarily favorable case for further analysis, since the following mutant genes were present in this region of the X chromosome: White and its 8 allelomorphs, one of which is eosin (at 1.1), facet (at 2.2) and the gene for the dominant sex-linked character abnormal abdomen (at 2.4). Two of these characters, viz., eosin and facet, were in addition sex-limited, both being much more extreme in males than in females.

On the basis of the working hypothesis mentioned, it could be predicted that notch₈ females, when crossed to males carrying the recessive sex-linked genes just spoken of, would give F₁ notch₈ daughters which, in spite of being heterozygous, would show the characters. By crossing notch₈ females to males carrying other sex-linked genes to the left and to the right of this region it would be seen in F₁ if other loci also were included in the deficient region, and a preliminary measure of the extent of the deficiency could in this way be obtained.

The result of these crosses proved the hypothesis to be correct. White and all its allelomorphs manifested themselves in the heterozygous F₁ notch₈ females; so also did facet. Abnormal abdomen, being partially dominant, would be expected to appear to some extent anyhow. Special facts with regard to the latter cross will be given later. None of the other

sex-linked genes (those to the left and right) showed in the F_1 notch₈ females. The gene nearest eosin to the left is the recessive broad (wings), (at 0.4), the one nearest to the right of the old notch is the recessive echinus (eye), (at 5.6). It could accordingly be concluded that the deficient region was not long enough to cover this distance, 5.2 units.

The examination of the F_1 notch₈ females heterozygous for the allelomorphs of white or for facet or abnormal abdomen revealed a new and important fact. The notch₈ flies heterozygous for an allelomorph of white had a lighter eye color than the flies in the corresponding stocks. This exaggerating effect of deficiency was especially striking in notch flies heterozygous for the darker allelomorphs of white, such as cherry, coral or blood. Similarly heterozygous notch₈ facet females had much more extreme facet eyes even than the facet males. Also the notch₈ females heterozygous for abnormal abdomen had a more marked abnormal abdomen than their heterozygous abnormal-abdomen sisters, though this difference was less pronounced than was the case with regard to facet. This result indicated that the deficiency exaggerates the effect of all the mutant genes present in the corresponding region of the other X chromosome.

To test this point under optimal conditions, deficiency notch₈ females heterozygous for white or the allelomorphs of white were back-crossed in pair matings to males from the white or to the corresponding white-allelomorph used in the cross. The not-notch females among the progeny were homozygous for the allelomorph of white used, and direct comparison was made between them and the eye colors modified by notch. The result of this experiment fully confirmed the earlier observation. The notch₈ flies always had lighter and more transparent eyes than their homozygous sisters. This effect of deficiency could be detected even in notch flies heterozygous for the lighter eye colors belonging to the white-allelomorph series.

An exaggerating effect of deficiency on other light eye colors was observed in other experiments. When the deficiency notch₈ was present in flies homozygous for the sex-linked genes vermilion and garnet, these eye colors were markedly lighter than in the not-notch sisters. Likewise notch₈ females homozygous for pink, the gene of which is in the third chromosome, showed a lighter eye color than homozygous pink flies. No similar effect on the darker eye color sepia in the third chromosome could be observed.

From these tests it was seen that deficiency notch₈ acted very much

like the factor for white itself. For, not only are compounds of white with its allelomorphs lighter than the later allelomorphs when homozygous, but the gene for white when present in heterozygous condition has also a diluting effect on some other eye colors, such as pink (MORGAN and BRIDGES 1913).

It was found, however, in the course of the above experiment, that deficiency seemed to have an even more pronounced effect than white itself was known to have. To ascertain this point, notch₈ females heterozygous for white were back-crossed in pair matings to males carrying the different allelomorphs of white. This would be a decisive experiment since the F₁ notch₈ daughters would have deficiency in one chromosome and the allelomorph of white in question in the other, while their sisters would have white in one chromosome and the same allelomorph in the other. The comparison could in this way be carried out under absolutely equal conditions, since the flies to be compared were raised in the same culture and derived from the same parents.

The result of this experiment proved the correctness of the above supposition. The deficiency-allelomorph compound was always somewhat lighter and more transparent than the corresponding white-allelomorph compound. Even in the lighter eye colors of the allelomorphic series the females could be separated on the basis of this difference in eye color. Deficiency notch₈ acts accordingly as a sub- or infra-white.

The marked exaggerating influence of the deficiency notch₈ on the effect of all the mutant genes located in the corresponding region of the other X chromosomes, could hardly be brought into accord with the earlier conception that deficiency represented a physical loss or a total inactivation. It was accordingly regarded highly desirable to be able to compare a notch₈ female heterozygous for one of the darker allelomorphs of white with a non-disjunctional XO male carrying the same allelomorph in its X chromosome. Such a male would show the effect, if any, of the total loss, since it has only one X chromosome, the entire Y chromosome being absent. No such effect in the XO males had previously been recorded, but much attention could hardly have been paid to this special point.

By chance, a non-disjunctional XO cherry male occurred in a cross between a notch₈ female and a cherry abnormal-abdomen male. This male could accordingly be compared with his own notch sisters heterozygous for cherry. That the male in question really was of the above non-disjunctional type could be ascertained by the fact that though tested

with six females, he proved to be sterile, since BRIDGES (1916) has demonstrated that non-disjunctional XO males are always sterile. This XO cherry male had the ordinary dark eye color of regular cherry males, much darker than that of his heterozygous deficiency-cherry sisters.

An analogous exaggerating effect of deficiency was, as mentioned, observed with regard to facet. Heterozygous facet-notch₈ females have a rough eye much more extreme than that of facet males, which in turn show the facet character much more pronounced than homozygous facet females. Moreover, the wings of the heterozygous notch-facet females were always very much modified, being constantly very markedly notched at the ends and in addition along the sides, so that the whole wing had a spade-like form. These squared-off wings were in addition extended, forming an angle with each other. When the facet stock was looked over it was observed that facet flies showed a tendency to a slight notching of the ends of the wings, most frequent in the males. The modified wing of heterozygous notch₈-facet females was accordingly regarded as an expression of this tendency to notching produced by the ordinary facet gene, exaggerated, in this case, by its combination with the deficiency notch₈.

With regard to the notch₈ × abnormal-abdomen crosses it should be noticed that the dominant character abnormal abdomen demands special conditions in the culture bottle to manifest itself (MORGAN 1915). It would be regarded *a priori* as fairly probable that the deficiency, exaggerating the effect of the genes in the opposite region, would cause a dominant character of this type, which had its gene within this region, to manifest itself in F₁ notch₈ flies heterozygous for abnormal abdomen, even if the culture conditions were not favorable for a general manifestation of the character. This was found, however, not to be the case. Several crosses were made up by mating notch₈ females to abnormal-abdomen males in which cultures the latter character failed to show in F₁ either in the notch or in the not-notch females. In later experiments, however, where the attempts to create the special conditions necessary for the development of abnormal abdomen were successful, it was found that notch females generally had a more marked abnormal abdomen than their heterozygous, not-notch sisters.

Summing up, we find that the deficiency notch₈ resembles the earlier described bar-deficiency in the fact that, when it is present, recessive genes in that region which is homologous to the deficient region manifest themselves when present in heterozygous condition. Like the bar-deficiency, too, this new deficiency also acts as a lethal in the males.

But in addition to allowing them to show, this new deficiency exaggerates the effect of all known mutant genes located in the corresponding region of the opposite X chromosome. This effect was not observed in the case of bar-deficiency.

The analysis of bar-deficiency led to the conclusion that deficiency was due to a physical loss or a total inactivation of an entire region of a chromosome. These alternatives, loss or inactivation, can hardly be maintained as an explanation of the case of deficiency here described. This deficiency is shown to have a striking general effect on all mutant genes in the corresponding region of the other X chromosome. It would be very difficult to conceive that this effect could be caused by a total loss or a complete inactivation, for it has been demonstrated that the total absence of one sex chromosome in a non-disjunctional XO male (which contains not even a Y chromosome) had not the slightest effect upon a mutant character (cherry) which was markedly changed by the deficiency notch₈.

Moreover, ordinary males (XY) carrying one of the allelomorphs of white are all, except eosin, of just the same eye color as females homozygous for the same allelomorph.

Thus, if the deficiency were regarded as a total loss or a complete inactivation we should be confronted with the situation that, whereas a single gene in the female gives the lighter eye color seen in the heterozygous notch₈ flies, two genes together in the female give an eye color exactly like that of the male carrying one gene. This would be a too strange coincidence when we remember that it applies to six genes of very different grades of eye color.

It has been shown that deficiency notch₈ exaggerates the effect of all the known mutant genes located in the corresponding region of the other X chromosome. The visible result is that flies carrying the deficiency in one chromosome and one of these genes in the other are further removed from normal, than is the case in homozygous females or males carrying the same gene.

The question arises accordingly if normal genes in the region opposite to the deficient piece are not also influenced in a similar way.

To some extent this seems to be the case. The series of somatic abnormalities found in the notch₈ flies points in this direction. The notch₈ flies are different from wild-type flies in the following respects: The wings are irregularly nicked at the ends, and often somewhat extended forming an angle with each other; certain veins are thickened; the eyes

are generally small and show a slight tendency to roughening; the acrostical hairs are irregular in their distribution, not arranged in definite rows as is the case in the wild-type fly; extra scutellar bristles are apt to appear.

It is natural to suppose that these somatic peculiarities are a result of the modification of the effects of one or more of the normal genes in the region opposite to the deficient piece, similar to that which has been demonstrated in the case of the mutant genes. It is superfluous to regard the character notch as due to an independent specific mutant gene contained in or linked to the deficient region.

It would seem probable that many normal genes are contained in such a piece of the X chromosome as that opposite to the deficient region. The fact that more extensive alterations are not caused when the deficient chromosome is present could perhaps be said to point in the direction that the normal genes must have different potencies. The mentioned mutant genes and some of the normal ones in the region opposite are affected, while other normal genes, supposedly present in the same region, are not visibly influenced.

In connection with the description of this case of deficiency the remarkable fact might be recalled that a majority of the known dominant genes in *Drosophila* are lethal when homozygous, like yellow body color in mice. Notch itself was in fact previously placed in this group (MULLER 1917). The notch₈ case is the first in which an explanation of this peculiar relation has been possible. It might very well be that some of the not-sex-linked cases of this type are also due to deficiency. The lethal effect of the genes in homozygous condition is just what was to be expected if this were true. Deficiency may be a more common phenomenon than so far regarded probable. If the region opposite to the deficient piece did not contain mutant genes or normal ones which are modified in their effect in the presence of the deficiency, the deficiencies would not be recorded as such, but only as lethals. Some of the numerous lethals in *Drosophila* may be due to such deficiencies.

The data obtained concerning the effect of deficiency notch₈ on the process of crossing over will not be presented here. It should only be said that no crossing over takes place within the deficient region. The presence of deficiency seems also to influence in a special way the amount of crossing over in the neighborhood of the deficient region. Preliminary linkage tests indicate that the deficient region extends somewhat to the left of eosin and considerably to the right of facet, in fact to within

0.7 unit of echinus, making at total length of about 4.8 units. This indicates that the extent of the notch₈ deficiency is so considerable that a cytological examination might throw light on the physical nature of deficiency.

It should be mentioned that of the previously recorded notch mutations two were tested in crosses to facet and proved to be deficiencies for this locus (see above). Five were used in experiments in which they were crossed to white or its allelomorphs, but these genes did not manifest themselves when heterozygous in the notch flies. Deficiencies in this region of the X chromosome may accordingly differ in length and still give the notch character.

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THE INHERITANCE OF THE MUTANT CHARACTER "VORTEX"

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INTRODUCTORY

There seems to be an impression that the great majority of mutants of *Drosophila* are comparatively simple in their genetic behavior. Because of their superior usefulness the mutants whose inheritance is clean-cut have been practically the only ones employed in the experiments for the analysis of genetic phenomena. Other mutants occur, and not infrequently, which must be made the objects rather than the tools of investigation. In the following paper is given an account of such a character, "vortex."

ORIGIN OF THE VORTEX CHARACTER

In looking over the "California wild" stock of *Drosophila melanogaster* in November 1913, an occasional fly was found which showed on the thorax a pair of "rosettes"; that is, in the areas lateral to the dorso-central bristles the microchaetae or small hairs were arranged in a pair

of whorls. Specimens of this character were noticed on other occasions, but no breeding work was done until August 7, 1916, when a female was found that had in addition to the posterior rosettes an anterior pair. This female gave rise to the stock "vortex," with which the present work has been done.

DESCRIPTION OF THE VORTEX CHARACTER

In appearance and in degree of development the vortex character is quite variable. In the modal condition (figure 1*b*) two brown-pigmented spots are present, located lateral to and midway between the anterior and posterior dorso-central bristles. The pigment lies in the walls of an indentation or funnel that extends more or less deeply into the thorax. The microchaetae for a considerable area around this focus are arranged in a whorl. Also the dorso-central bristles, especially the anterior pair, are involved in the whorled formation.

In more pronounced specimens (figure 1*c*) these vortices are much more conspicuous and the central funnel is partly evaginated like the

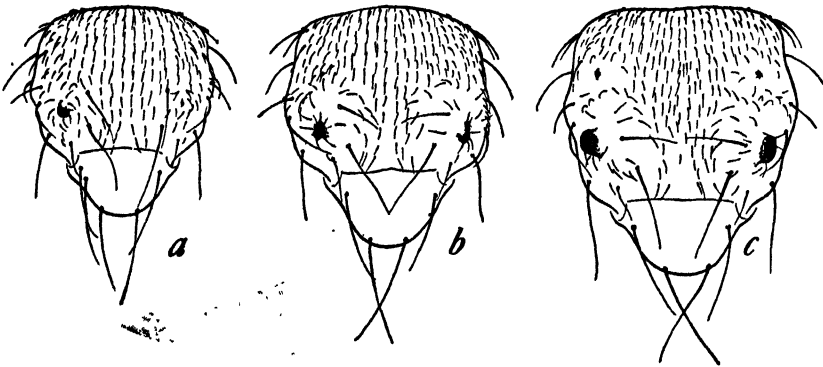


FIGURE 1.—The mutant character vortex. *a* represents a "slight" type (δ) with no anterior vortices; *b* represents the "modal" type (♀) with conspicuous funnel-like vortices; *c* represents an "extreme" type with everted funnels, and an anterior pair of vortices. *a* shows a symmetrical development that is fairly common.

finger of a glove, or even wholly evaginated into a horn-like elevation. In these extreme specimens an anterior pair of whorls is also developed. These anterior vortices are never as pronounced as the posterior ones.

In the "slight" cases (figure 1*a*) only the rosette-like arrangement of the hairs, or only a small brown pigmented depression indicates the character. A very small proportion of the individuals, genetically the same as the others, entirely fail to show the character, and are somatically normal.

All intergrades between this complete absence of the character and its fullest expression are met with in the same culture, although the bulk of the individuals are of the "modal" type.

In general the degree of development is approximately the same on the two sides. But within all grades there may be asymmetrical development of the character, so that the anterior vortex on one side may be absent giving a tri-vortex condition; also uni-vortex individuals are not uncommon.

The character is sex-limited to a considerable degree; i.e., females exhibit a higher grade of the character than do the males. The slight grades are preponderantly males and those few individuals that fail to show the character are practically always males.

The vortex flies do not differ from wild flies in any other respect that we have observed, and they are of good viability.

THE BIGENIC NATURE OF THE VORTEX CHARACTER

The first test usually made with a mutant character is the determination of the chromosome to which its gene belongs. The most approved method for doing this is to cross the flies in question to flies carrying the dominant characters star and dichaete, star being in the second chromosome (at 0.0) and dichaete in the third (at 11.7). By means of back-cross tests of the F_1 star dichaete males it is possible to ascertain whether the gene for the mutation lies in the second or the third chromosome or whether it is independent of both. The star dichaete back-cross method takes advantage of the fact of no crossing over in the male. Thus, if the gene for the mutant in question is in the second chromosome all the back-cross progeny will fall into two classes, namely, those showing star and those showing the mutant, with a total absence of individuals showing both star and the mutant, or, conversely, neither.

Accordingly, the original vortex female was at once out-crossed to a star dichaete male. Apparently this female was non-virgin and had already been fertilized by a brother heterozygous for vortex, since of the F_1 offspring, 17 showed the character vortex while the remaining 61 did not show vortex but did show the star and dichaete in all combinations but with some excess in the wild-type class (culture 4955, Aug. 17, 1916).

The fact that criss-cross inheritance was not shown—that among the F_1 males none of the stars or dichaetes showed vortex—proved that the character is not sex-linked.

The fortunate presence of the vortex flies in the F_1 culture bottle gave immediate materials for making the desired back-cross test. Four back-cross cultures were raised from matings between sister vortex females and F_1 males heterozygous for star, dichaete and vortex.

TABLE I

P_1 mating, star dichaete male by vortex female; back-cross mating, vortex female by F_1 star dichaete male.

1916 Aug. 31	Vortex	Vor- tex star	Vor- tex di- chaete	Vor- tex star di- chaete	Wild- type	Star	Di- chaete	Star di- chaete
5056 ♀	16	—	—	—	2	11	12	16
♂	6	—	—	—	11	12	12	22
5077 ♀	12	—	1	—	—	18	10	21
♂	6	—	—	—	1	17	5	18
5078 ♀	18	—	—	—	27	11	13	26
♂	15	—	—	—	18	22	10	20
6126 ♀	13	—	1	—	13	2	9	9
♂	16	—	—	—	7	9	14	20
Total ♀	59	—	2	—	42	42	44	72
♂	43	—	—	—	37	58	41	80
Grand total	102	—	2	—	79	100	85	152

The first point observed was that none of the back-cross star flies showed the vortex character. This linkage between star and vortex indicated that a second-chromosome recessive was essential for the production of vortex. Aside from two female exceptions none of the dichaete flies was vortex; by the same reasoning it is then obvious that the production of vortex depends also upon the action of a third-chromosome recessive gene. That is, the vortex character is the product of the simultaneous action of two independent genes, one in the second and one in the third chromosome.

If the above hypothesis is correct the vortex class should appear as one quarter of the offspring of such a back-cross test. In fact, the vortex individuals totaled 104 out of 520 or 21.2 percent, which is a fairly close approximation to the 25 percent expected. Furthermore, taking account of the fact that there is no crossing over in the male the back-cross flies should be in the ratio 1 vortex: 1 star: 1 dichaete: 1 star dichaete, which is approximated in the observed ratio 102 vortex: 100 star

: 85 dichaete: 152 star dichaete, although the star dichaete class is too large.

There were two further points in which the results failed to agree with the simple explanation thus far suggested. Two dichaete vortex females occurred, which were explained by the assumption that occasionally an individual homozygous for vortex II and heterozygous for vortex III shows the character. Later work has confirmed this hypothesis, for it has shown that the vortex III gene is not strictly recessive in flies homozygous for vortex II. The second point is the occurrence of the large and unexpected class of wild-type flies. An explanation for some of these wild-type flies was obtained when vortex flies were bred together (table 2).

TABLE 2
Progeny from crosses of vortex female by vortex male.

1916, Sept. 20	Vortex ♀	Wild-type ♀	Vortex ♂	Wild-type ♂
5255	98	1	84	19
5271	99	1	95	6
5458	12	—	10	—
5466	2	—	5	—
5467	8	—	2	—
5468	10	—	2	—
5469	17	—	17	—
5676	55	1	45	13
5701	27	—	45	—
5702	37	—	44	5
5867	64	2	59	10
Total	429	5	391	53

In making the counts of table 2 it was apparent that especially in the males a considerable proportion (about 20 percent), showed the character to a very slight extent. It is probable that some of the so-called wild-type flies in the back-cross counts of table 1 would have shown the vortex in this slight form had they been classified with knowledge of this point. However, even with close examination certain flies of the "pure cultures" of table 2 failed to show the character. In this connection an interesting fact was noted; namely, that those few wild-type females which occurred were among the last to hatch. Thus, in cultures 5255, 5271, and 5676 the single wild-type females occurred in the last day's count. A similar phenomenon was noted with regard to the males. The first flies to hatch were all quite extreme vortexes. Somewhat later the

males were less extreme on the average, and an occasional wild-type male occurred. In those cultures in which a large number of wild-type males appeared the majority were in the latest counts.

But that the above points do not entirely explain the back-cross results is apparent when it is realized that in two of the cultures (5078, 6129) there were many wild-type females. It seems probable from later work that in these two cultures the vortex mother was of the type heterozygous only for the vortex III gene, in which case the number of wild-type and of vortex offspring should be about equal.

THE LOCI OF THE VORTEX GENES

The same mating which gave material for the above back-cross tests of the male, gave an opportunity to test at once the question of the localization of the two genes concerned. By crossing the F_1 star dichaete females from the cross of vortex by star dichaete, to vortex males the amount of crossing over between star and the second-chromosome gene, and at the same time the amount of crossing over between dichaete and the third-chromosome gene, could be found. Out of the more than twenty such cultures that were started only three were successful (table 3).

The results of the female back-crosses parallel quite closely those of

TABLE 3

P₁ mating vortex female by star dichaete male; back-cross mating, F₁ star dichaete female by vortex male.¹

1916 Aug. 28	v_o	S v_o	D v_o	S D v_o	+	S	D	S D
5047 ♀	16	—	8	I	2	15	7	11
♂	6	—	1	—	2	15	7	11
5254 ♀	3	—	2	—	1	10	—	9
♂	8	—	1	—	2	3	4	6
5866 ♀	11	—	2	—	2	5	17	13
♂	12	1	6	—	—	6	13	22
Total ♀	30	—	12	I	5	30	24	33
♂	26	I	8	—	4	16	29	42
Grand total	56	I	20	I	9	46	53	75

¹ The symbol for the dominant character star is the capital letter S ; that for the designated v_o ; the vortex gene situated in the second chromosome is v_{oII} , that in the third is v_{oIII} . The + sign is read "wild-type."

the male back-cross of table 1, except that apparent crossovers occurred. The small number of wild-type flies, especially males, in these cultures indicates that there was very slight changing over of vortex into wild-type, and it is probable that these wild-type flies, certainly the females, represent crossing over between star and vortex II. A calculation of the location of the second-chromosome vortex gene gave a star vortex cross-over value of about 10.

There was uncertainty with respect to the dichaete vortex class, since in the male tests of table 1, where there was no crossing over, there had appeared two such dichaete vortex females. A calculation made on the uncertain basis that the dichaete vortex flies were crossovers gave a total of 21 vortex dichaete among the 78 vortex or 27 percent of crossing over. It was accordingly decided as possible that the third-chromosome gene for vortex lies to the right of dichaete at a position near to the center of the third chromosome.

It had been hoped to get far more adequate data with respect to the location of the second- and third-chromosome genes. But an unexpected obstacle presented itself. It was only with the greatest difficulty that the cultures involving vortex could be reared. The back-cross tests of the female were started on a large scale but of the more than twenty cultures all but three proved sterile, and these three produced relatively few flies. The same low productivity had been apparent in some of the pair cultures of vortex by vortex of table 2.

From the preceding considerations it was apparent that several points were capable of further elucidation. The occurrence of the class of wild-type flies in the back-cross tests of the male (table 1) was not accounted for beyond question. The appearance of dichaete vortex flies, which simulated crossovers in the male back-cross test, had received an explanation requiring experimental tests. It seemed possible that sterility was in some way connected with the above aberrations. Furthermore, a case ("pale") had arisen the characteristics of which gave a suggestive parallelism with the vortex case, namely, simultaneous linkage to both the second and the third chromosome, the appearance of unexpected classes, and of lethal effect. The analysis of that case had led to the hypothesis that a piece of the second chromosome had been removed and had been attached to the middle of the third chromosome. The removal of the piece of the second chromosome (deficiency) gave the effect of a lethal located in the second chromosome. The attachment of that piece to the third chromosome (duplication) explained the linkage of the contained

genes to the third chromosome genes. The place of attachment of this "transposed" piece was apparently at the middle of the third chromosome, which is the place of the spindle-fiber attachment. If the simultaneous linkage of vortex to the second and to the third chromosome was based on some such "transposition" then the transposed piece should likewise be attached to the spindle fibre and the linkage of vortex should correspond to a locus at the middle of the third chromosome. The preliminary calculation had suggested that this indeed was the case since there seemed about 27 percent of crossing over between *dichaete* and *vortex*, *dichaete* being known to be some 25 units from the center of the chromosome. A test of the above points demanded first of all a repetition of the original experiments on a larger scale and with close attention to the questionable features.

TESTS OF THE WILD-TYPE FLIES OF VORTEX STOCK

The first point tested was the assumption that the wild-type flies that occurred in the stock of *vortex* were simply fluctuants and were of the same genetic constitution as those flies which showed the character. A pair of such wild-type flies (M251) gave 62 *vortex* individuals and only two wild-type individuals, which were males. This was an entirely regular result comparable with the progeny given by *vortex* pairs. A second pair (M312) gave 71 females all of which were *vortex* and only four wild-type males among the 65 males of the culture. A pair of extreme *vortex* individuals mated at the same time gave 91 *vortex* and 13 wild-type individuals (M236). From these tests it is apparent that flies of the stock may give the same results irrespective of their grade, that is, of their somatic appearance.

REPETITION OF THE MALE BACK-CROSS TEST WITH STAR *DICHAETE*

The second test was a repetition of the male back-cross which had given the numerous wild-type flies. Ten such cultures were raised with no sterility (table 4).

During the classification of the flies of table 4 particular attention was paid to the classes which had caused confusion before, namely the wild-type and the *dichaete vortex* classes, in order that none of the flies classed as wild-type should show *vortex* even slightly, and likewise that all of the *dichaete* flies which showed the *vortex* might be separated out.

The first point which appeared in the new cultures was that the number of wild-type flies in no case exceeded the number which could read-

TABLE 4

*P*₁ mating, vortex female by star dichæte male, back-cross mating, vortex female by *F*₁ star dichæte male.

1918 July 9	<i>v</i> ₀	<i>S</i> <i>v</i> ₀	<i>D</i> <i>v</i> ₀	<i>S</i> <i>D</i> <i>v</i> ₀	+	<i>S</i>	<i>D</i>	<i>S</i> <i>D</i>
M337 ♀	21	—	—	—	—	20	18	39
♂	20	—	—	—	3	21	16	28
M338 ♀	18	—	—	—	—	21	23	15
♂	10	—	—	—	9	10	11	12
M339 ♀	28	—	7	—	—	26	17	22
♂	12	—	—	—	1	35	25	24
M340 ♀	15	—	—	—	—	23	18	30
♂	21	—	—	—	1	18	22	23
M341 ♀	21	—	3	—	—	31	14	20
♂	20	—	—	—	2	18	23	26
M347 ♀	29	—	3	—	—	30	19	28
♂	25	—	—	—	2	21	22	26
M348 ♀	26	—	3	—	—	21	15	19
♂	23	—	—	—	1	34	20	29
M350 ♀	12	—	1	—	—	14	13	16
♂	10	—	—	—	—	15	11	18
M354 ♀	18	—	8	—	—	17	10	12
♂	17	—	—	—	3	15	14	22
M356 ♀	26	—	2	—	—	17	20	26
♂	20	—	—	—	1	21	20	27
Total ♀	214	—	27	—	—	220	167	227
♂	178	—	—	—	23	208	184	235
Grand total	392	—	27	—	23	428	351	462

ily be explained by the amount of overlap of vortex into normal. This wild-type class was further characterized by being entirely confined to the males, not a single wild-type female having occurred. As had been discovered in the case of the wild-type fluctuants in the vortex stock these wild-type males were in general restricted to the late counts.

The sums of the wild-type and the vortex flies gave a class of the same size as each of the other expected classes and not a large excess as had been the case in the two exceptional cultures of table 1. Definite proof in the case of two of the wild-type flies that they were genetically vortex was furnished by crossing them to vortex females from stock. One of the tested males gave 62 vortex females, 79 vortex males and only three wild-types which were males (M431). The other gave 32 vortex females and 21 vortex males with no wild-type flies (M440).

The apparent crossover class of dichæte vortex reappeared in the new

experiment, but was entirely confined to the females. It had been suggested on the basis of the former experiments that these *dichaete* vortex flies were not homozygous for both vortex genes, but were homozygous for the second-chromosome gene and only heterozygous for the third-chromosome gene. If this were true such flies should give, when tested by homozygous vortex males, about half of the offspring vortex and half not vortex. Were they really homozygous for both genes, almost all of the offspring ought to be vortex as in a stock culture. One such vortex *dichaete* female (from M354) was accordingly tested by crossing to vortex males from stock. The offspring (M396) were: v_0° 29, $+$ 0, D° 41, Dv_0° 1, v_0^{δ} 26, $+$ 6, D^{δ} 39, Dv_0^{δ} 0. That is, the *dichaete*-bearing third chromosome did not carry the vortex III gene, and approximately half of the flies were vortex instead of nearly all being vortex. This result proved that it is possible for a female only heterozygous for the third-chromosome vortex gene to show the character when homozygous for the second-chromosome gene. Furthermore the single *dichaete* vortex female which occurred in the above test culture was of the same constitution as her mother as was proved by the progeny obtained by crossing her to a vortex male from stock. The progeny were: v_0° 76, $+$ 0, D° 70, Dv_0° 15, v_0^{δ} 83, $+$ 3, D^{δ} 83, Dv_0^{δ} 0, among which progeny the vortex constituted 174 out of 330 flies.

Since the suggested explanation has proved to be correct, an interesting comparison between this *dichaete* vortex and the wild-type class presents itself. All the wild-type flies were male, but the *dichaete* vortex flies were without exception female. While this is apparently an inverse relation it is in reality an expression of a single phenomenon—the partial sex-limitation of the character. This sex-limitation permits a readier and more marked expression of the character in the female than in the male, both in the double homozygous condition and also in the special case of the heterozygote just considered.

LOCATION OF THE VORTEX GENES THROUGH LINKAGE TESTS WITH STAR AND DICHAETE

The first experiment to locate the vortex genes more accurately was a repetition of the female back-cross test with star and *dichaete* (table 5).

The results to be expected from the female back-cross are much more complex than those that form the male tests; in addition to the changing over of vortex into wild-type (δ^{δ}) and the presence of supernumerary vortexes of the heterozygous type (\circ°), in the case of the female test

TABLE 5
*P*₁, vortex ♀ × star dichæte ♂; *F*₁ star dichæte ♀ × vortex ♂.

1918 July 14	<i>v</i> ₀	<i>S</i> <i>v</i> ₀	<i>D</i> <i>v</i> ₀	<i>S</i> <i>D</i> <i>v</i> ₀	+	<i>S</i>	<i>D</i>	<i>S</i> <i>D</i>
M359 ♀	34	10	7	1	2	30	26	35
♂	36	3	—	—	5	32	32	27
M366 ♀	34	1	1	—	5	38	47	44
♂	28	4	—	—	8	37	48	43
M367 ♀	36	5	13	2	7	28	39	38
♂	40	2	—	—	3	33	48	59
M368 ♀	40	3	8	1	3	41	29	52
♂	38	2	—	—	8	44	60	37
M397 ♀	27	2	10	2	7	26	40	35
♂	37	3	—	—	5	27	39	32
M398 ♀	16	1	7	5	2	11	15	24
♂	16	2	—	—	2	17	16	13
M399 ♀	44	—	10	2	2	36	29	41
♂	31	7	—	—	8	37	42	35
M400 ♀	32	5	11	—	5	39	26	35
♂	35	2	—	—	9	36	36	39
M401 ♀	22	3	6	1	4	17	26	27
♂	27	1	—	—	3	13	27	20
M402 ♀	31	2	1	—	2	26	25	33
♂	38	7	—	—	5	27	27	31
M425 ♀	22	2	—	—	2	13	11	26
♂	8	1	—	—	1	13	13	19
M426 ♀	23	4	1	—	2	29	32	34
♂	37	5	—	—	6	35	45	39
M427 ♀	42	2	—	—	2	36	30	41
♂	29	2	—	—	3	37	36	47
M428 ♀	31	—	—	1	4	45	29	40
♂	41	4	—	—	8	40	35	32
M429 ♀	37	4	2	—	3	28	41	36
♂	19	4	—	—	4	33	57	37
Total ♀	471	44	77	15	52	443	445	541
♂	460	49	—	—	78	461	555	510
Grand total	931	93	77	15	130	904	1000	1051

crossing over gives classes identical in appearance but different in their genetic origin. Thus the non-vortex classes are each composed, theoretically, of progeny from three sources according to whether they represent crossing over in the second, the third, or in both the second and the third chromosome, as may be seen from table 6.

A fortunate simplification of this problem is obtained from a con-

TABLE 6

Classes of eggs produced by a female of the type, $\frac{S}{v_{oII}} \frac{D}{v_{oIII}}$, and of offspring when such eggs are fertilized by sperm of a vortex male.

Non-crossover (A)				Crossover in II (B)				Crossover in III (C)				Crossover in II and III (D)			
$\frac{S}{D}$	$\frac{v_{oII}}{v_{oIII}}$	$\frac{S_o}{v_{oIII}}$	$\frac{v_{oII}}{D}$	$\frac{Sv_{oII}}{D}$	$\frac{+}{v_{oIII}}$	$\frac{Sv_{oII}}{v_{oIII}}$	$\frac{+}{D}$	$\frac{S}{Dv_{oIII}}$	$\frac{v_{oII}}{+}$	$\frac{S}{+}$	$\frac{v_{oII}}{Dv_{oIII}}$	$\frac{Sv_{oII}}{Dv_{oIII}}$	$\frac{+}{+}$	$\frac{Sv_{oII}}{+}$	$\frac{+}{Dv_{oIII}}$
$\frac{S}{D}$	$\frac{v_o}{v_o}$	$\frac{S}{S}$	$\frac{D}{D}$	$\frac{S}{D}$	$\frac{+}{+}$	$\frac{S}{v_o}$	$\frac{D}{D}$	$\frac{S}{D}$	$\frac{v_{oII}}{+}$	$\frac{S}{S}$	$\frac{D}{v_o}$	$\frac{S}{D}$	$\frac{+}{+}$	$\frac{S}{S}$	$\frac{D}{D}$
												$\frac{v_o}{D}$			
Vortex = A =				♀ ♀	♂ ♂					Wild-type = B+C+D =				♀ ♀	♂ ♂
Star vortex = B =				471	460					Star = A+C+D =				52	78
Dichaete vortex = C =				44	49					Dichaete = A+B+D =				443	461
Star dichaete vortex = D =				77	—					Star dichaete = A+B+C =				555	510
				15	—										

sideration of the dichaete and the star dichaete classes. Among the males not a single vortex individual of these classes appeared. Since the changing over of genetically vortex into somatically not-vortex is very slight throughout this experiment, this complete absence of males of these classes means that none or only a negligible amount of crossing over occurred between dichaete and the third-chromosome vortex gene.

We must omit from our calculation of the amount of crossing over between dichaete and vortex III the data from the females, since in the females it is known that individuals simply heterozygous for vortex III ($\frac{v_{oII}}{v_{oII}} \frac{v_{oIII}}{+}$) in some cases show the vortex character. In fact if crossing over between dichaete and vortex III is as rare as is indicated by the male data then all or practically all of the dichaete vortex and star dichaete vortex females were of this heterozygous type. Two of these dichaete vortex and two star dichaete vortex females were tested by crossing to vortex males, and in all cases they proved to be of the heterozygous type (tables 7 and 8).

The complete absence of dichaete vortex males in table 5 is in contrast with their occurrence in the similar experiment of table 2. Instead of quite free crossing over between dichaete and vortex III, as at first supposed to be the case, there is no good evidence of any crossing over at all between them. The dichaete vortex males of table 2 must then have been due to another cause, and certain similar results, to be described in a later section,

TABLE 7

Tests by vortex males of dichæte vortex females from the female back-crosses of star dichæte by vortex.

	v_o	+	D	$D v_o$
M445 (ex 397) ♀	60	—	38	32
♂	68	—	87	—
M446 (ex 397) ♀	78	—	45	30
♂	88	1	93	1

TABLE 8

Tests by vortex males of star dichæte vortex females from the female back-crosses of star dichæte by vortex.

	v_o	$S v_o$	$D v_o$	$SD v_o$	+	S	D	SD
M447 (ex 398) ♀	51	41	2	3	—	—	30	27
♂	37	40	—	—	—	—	32	31
M462 (ex 398) ♀	29	16	9	10	—	—	16	14
♂	26	28	—	—	—	—	31	23

will make it apparent that this cause may have been an additional semi-dominant modifier.

With respect to the linkage between star and the second-chromosome vortex gene, the crossover classes are star vortex and wild-type. When there is considerably more changing over in the male classes than occurred in this experiment there is still no changing over in the females. Four of the wild-type females were tested, and as expected, in no case were they vortexes that had changed over (table 9, cultures 412, 438, 448, 454). The 52 wild-type females of table 5 are therefore all to be considered as true crossovers between star and vortex II. The non-crossover class which corresponds to this wild-type crossover class is the star class of 443 females. Star vortex (44) and vortex (471) are complementary crossover and corresponding non-crossover classes.

Because of the probability of a slight amount of changing over among the males the wild-type males (78) can not be used without correction, which is here of doubtful validity. Of three such wild-type males tested, one was a true wild-type crossover, but the other two were changed over vortex non-crossovers (table 9, cultures M437, M452 and M460).

Males do not show vortex unless they are homozygous for both genes or contain an additional modifier, and all vortex males of table 5 can therefore be used in the calculation. The star vortex class (49) is the

TABLE 9
Tests of wild-type flies from table 5 by out-crosses to vortex.

No.	From	Vortex ♀	Wild-type ♀	Vortex ♂	Wild-type
M412 (♀)	M367	24	16	23	25
M438 (♀)	M367	92	113	109	101
M448 (♀)	M397	84	76	79	93
M454 (♀)	M400	80	72	82	73
M437 (♂)	M366	5	9	13	20
M452 (♂)	M402	136	—	104	20
M460 (♂)	M402	84	—	42	24

crossover and the vortex class is the corresponding non-crossover (460). The total number of crossovers available is 145 ($52 + 44 + 49$), and the corresponding total of non-crossovers is 1374. The percentage of crossing over between star and vortex II is therefore 9.5. The locus of star is so far to the left in the second chromosome that with a distance of nearly ten units between the star and the vortex loci it seemed far more probable that the locus of vortex II is to the right of star.

THE LOCALIZATION OF VORTEX II BY AID OF STREAK

If, as calculated, the locus of vortex II is about ten units to the right of star then the position of the gene could be more accurately obtained by means of the linkage relations of vortex with the dominant mutant streak. The locus of streak was known to be at about 14.7 units to the right of star, although the data on which that location was based was rather meager in amount. The locus of vortex II was therefore considered to be about five units to the left of that of streak. The most advantageous type of back-cross is that known as "alternated" in which the middle mutant gene is in one chromosome and the two end genes in the other $\left(\frac{S}{v_o} \frac{S_k}{v_o}\right)$. In order to obtain heterozygous females

of the required type a crossover star vortex male was taken from the previous experiment and crossed to streak females from stock (M439). The F_1 star streak females were then back-crossed by vortex males (M487). The small proportion of star streak crossovers which occurred among the back-cross offspring were of two types, half were only heterozygous for vortex III while the remaining half were of the desired homozygous type. To eliminate all doubt as to the constitution of the flies used some of the star streak crossovers were tested individually by mat-

ing to vortex females. One of these cultures (M520—SS_k♀ 69, ♂ 79; v_o♀ 74, ♂ 76; + ♂ 1) of which the father proved to have been homozygous for vortex III gave many star streak offspring, all of which were homozygous for vortex III and heterozygous for vortex II. Fifteen cultures were raised from star streak females of the above constitution (table 10).

TABLE 10
Back-cross tests of $\frac{S}{v_{oII}} \frac{S_k}{v_{oIII}} \frac{v_{oIII}}{v_{oIII}}$ ♀ by vortex ♂.

1918 Oct. 14	S S_k	v_o	S v_o	S_k	S	v_o S_k	S S_k v_o	+
M572 ♀	30	28	4	2	—	—	—	—
♂	25	26	2	3	1	—	—	2
M573 ♀	23	23	1	2	—	—	—	—
♂	21	14	3	1	2	—	—	—
M574 ♀	37	50	3	9	1	—	—	—
♂	57	42	3	5	3	—	—	2
M575 ♀	40	36	8	9	1	—	—	—
♂	44	46	2	7	3	—	—	6
M578 ♀	50	53	6	10	2	—	—	—
♂	42	65	3	5	5	—	—	7
M579 ♀	54	61	10	7	2	—	—	—
♂	50	48	4	8	3	—	—	8
M586 ♀	41	41	6	4	—	—	—	—
♂	34	24	5	2	—	—	—	1
M587 ♀	45	55	9	6	3	—	—	—
♂	41	45	5	8	4	—	—	8
M588 ♀	36	40	2	5	3	1	—	—
♂	43	33	7	6	2	—	—	2
M589 ♀	22	31	4	6	—	—	—	—
♂	22	31	3	5	2	—	—	1
M590 ♀	40	43	4	4	2	—	—	1
♂	44	40	3	10	4	—	—	1
M592 ♀	35	32	6	4	2	—	—	—
♂	38	32	2	7	4	—	—	13
M593 ♀	43	38	2	6	—	—	—	—
♂	23	26	2	2	2	—	—	—
M594 ♀	32	34	2	6	3	—	—	—
♂	30	40	5	6	3	—	—	10
M610 ♀	28	44	3	8	1	2	—	—
♂	27	26	5	14	—	—	—	4
Totals ♀	556	609	70	88	19	3	—	1
♂	541	538	54	89	38	—	—	65
Grand totals	1097	1147	124	177	57	3	—	66

The classification of the experiment of table 10 was safeguarded by isolating all doubtful flies for at least five days until the pigment of the streak and of the vortex characters was fully developed and until the bubbles (to be mentioned later) characteristic of streak become pronounced. In certain cases the further precaution of actual test matings was taken so that the separations as recorded in table 10 can be regarded as complete. Three males from M593 that were regarded as possible streak were tested but proved to be non-streak. The same result was obtained from tests of several of the vortex males wherever there was suspicion that they might be streak.

Before making the calculations of the amount of crossing over it is necessary to consider the changed-over classes. Extensive experiments involving this region of the second chromosome have shown that the amount of double crossing over within this distance of fifteen units is practically zero, so that there should be no wild-type class. It is doubtful whether the one wild-type female which occurred was such a double crossover or was a changed-over vortex. It seems more probable that she was a vortex female since she occurred in the last count of the culture. We may therefore add this one to the 609 vortex females. Likewise the 65 wild-type males (four of which were tested and proved to be vortex genetically) are to be added to the 538 vortex males bringing the number up to 603 which is then equal to the number of females. Among the males 10.8 percent of the vortex class changed over. If this same proportion of star vortex males changed over then six males should be transferred from the star class to the star vortex class, reducing the star class to 32 and increasing the star vortex to 60.

While the counts of table 10 were being made a striking fact was observed, namely, that the streak vortex class was practically non-existent, although it had been expected to be as large as the star class. Furthermore the vortex present in the three streak vortex flies recorded as such in table 10 was of a different type from the ordinary vortex, being developed only in the anterior pair of vortices as very slight depressions with little pigment and no whorling of the hairs. Morphologically there seemed some slight reason why the presence of the streak character should interfere with the development of the vortex character. The thorax of streak flies is markedly altered, especially with regard to the musculature, which is largely replaced by large bubbles. In fact this character of the thorax is the clearest one for classification. It is to be noticed that the anterior pair of vortices would

most often escape the interference by these alterations since the anterior pair of vortices is broadly separated laterally, while the center of the streak disturbance is median and posterior.

Tests, that will be described in a later section, were carried out with these particular streak vortex flies and these tests showed that there was probably present a modifier which favored the development of a vortex among streak flies of these cultures and their descendants.

Not only was the streak vortex class unduly diminished, but correspondingly the streak class was unexpectedly large, being 177 flies while its complementary class star vortex was only 130 flies (corrected for changing over). If the streak vortex flies were included with the streak then we should expect that the number of streak and streak vortex flies should be equal to the sum of the star and the star vortex flies. This is found to be the case, since the sum of the streak flies is 180, while the sum of the star flies is 181. There are two ways of calculating how many flies should be removed from the streak class and added to the streak vortex class. The class of streak vortex should be equal to the complementary class star which is 51 (corrected). This required the transference of 48 flies from the streak to the streak vortex class. The other method is to reduce the size of the streak class to that of its complement (130). This would require the transference of 50 flies from the streak to the streak vortex class. Since these two methods agree the corrected classes may be accepted as 129 streak and 51 streak vortex. The final corrected classes stand as in table II.

TABLE II
The classes of table 9 corrected for changing over and for interference by streak.

S	v_o	S	S_k	S	v_o	S	v_o	S_k	$+$
S_k		v_o		S	S_k	S	S_k	$+$	
1097	1213	129	130	51	51	—	—	—	—

On this basis there was 9.7 percent of crossing over between star and vortex, which is in agreement with the value (9.5) obtained from the star dichæte vortex female back-cross tests of table 5. There was 3.8 percent of crossing over between vortex and streak, which is slightly less than that previously calculated from data less extensive. The locus of streak on the basis of the entire data is at about 13.7 units to the right of star.

THE STREAK VORTEX MODIFIER

One of the two exceptional streak vortex flies which occurred in culture 610 was out-crossed to vortex males and gave a considerable proportion (about a quarter) of streak vortex flies of this new type (culture 694, table 12). A streak vortex male from among this progeny out-crossed to vortex females from stock likewise gave this type of streak vortex in the same proportion (M782). This stock has been continued for several generations and gives analogous results. The proportion of streak flies showing vortex in these two cultures and the line descended from one of them is exceptional, since in the other cultures the

TABLE 12
Selection for vortex streak (new type).

No.	Parentage		S_k	v_o S_k	v_o	+
M694	$v_o S_k \text{♀ ex } 619 \times v_o \text{♂}$	♀	23	17	40	—
		♂	19	6	44	8
		♀	31	8	40	2
M812	$v_o S_k \text{♂ ex } 694 \times v_o \text{♀}$	♂	17	2	36	7

crossover streak flies which are genetically homozygous for vortex fail to show the vortex character. The new condition which has arisen is probably due to a mutant modifier which has the effect of causing the vortex in streak flies to develop but to develop as a new somatic type. Since this type occurred in two cultures and in out-crossed cultures of their descendants, the modifying gene is a dominant. In the mother of culture 610 this dominant modifier was present in the star streak chromosome not far from streak and probably to the right. When crossing over occurred between vortex II and streak, the streak individuals received the modifier and were then better able to show vortex, but in a modified type. This accounts for the individuals in cultures 610 and 588. When a vortex streak crossover female from 610 was out-crossed, most of the streak descendants should be of the same constitution as the mother, that is, homozygous for vortex II and vortex III and heterozygous for streak and for the dominant modifier. The result showed that only about a quarter of the streak flies developed the new type of vortex. It might have been supposed that crossing over between streak and the modifier had reduced the number of flies containing the modifier and hence showing the new vortex. But in the next generation a

male of this constitution was out-crossed and results similar to those of the female out-cross were obtained. Since there is no crossing over in the male the similarity of the female and male out-crosses shows that in the female likewise there was probably little crossing over between streak and the modifier. It is evident then that this modifier is able to bring the new vortex to expression in only about a quarter of the flies of the given constitution.

There are many other cases known in which flies of a given constitution may or may not show a certain character. The determining factor is presumably environmental, and has been proved to be such in several of the cases.

FURTHER TESTS OF THE POSITION OF VORTEX III

The other experiments had indicated that the position of vortex III was very close to that of *dichaete*. In fact no certain crossover had been obtained between these two loci. It was thought advisable to get more extensive data on this point in the hope of finding on which side of *dichaete* the locus of vortex III is situated. Such an experiment would require the simultaneous use of two known loci in the third chromosome. The stock containing the two dominants *dichaete* and *hairless* offered the quickest and most convenient method of obtaining such information. Accordingly a vortex male was crossed to a *dichaete* *hairless* female and the F_1 *dichaete* *hairless* females heterozygous for vortex II and for vortex III were tested by vortex male from stock (table 13(A)). As in the previous experiment, females that were not homozygous for vortex III showed the vortex character occasionally. Thus among the *dichaete* females of table 13 (A) 16 showed vortex slightly. Tests of one of the vortex *dichaete* *hairless* females showed that it was the supposed heterozygous type (table 13 (C), culture M450). Such vortex *dichaete* flies likewise occurred in two parallel tests of F_1 males, and since no crossing over occurs in the males the vortex *dichaete* flies are clearly of the heterozygous vortex III type (table 13 (B)). For this reason it is only among the *dichaete* males of table 13 (A) that real crossing over could be detected. No such *dichaete* vortex males occurred, which confirms the closeness of vortex III to *dichaete*, but fails to show the relative order. On the other hand the crossing over between vortex and *hairless* was of the amount (20.4) to be expected from the known normal distance between *dichaete* and *hairless*.

TABLE 13
 (A) Back-cross tests of $\frac{+}{v_{011}} \cdot \frac{D}{v_{0111}} H$ female by vortex male.

1918 July 24		v_0	v_0	$\frac{v_0}{H}$	$\frac{D}{v_0 H}$	+	D	H	$\frac{D}{H}$
M389	♀	22	—	5	3	21	11	5	43
	♂	12	—	4	—	37	16	9	48
M390	♀	27	—	5	1	36	11	10	37
	♂	21	—	3	—	57	9	8	62
M421	♀	29	—	11	7	41	27	15	61
	♂	24	—	7	—	40	20	13	72
M422	♀	41	—	4	2	38	16	8	74
	♂	30	—	12	—	45	24	7	78
M423	♀	31	—	11	3	40	23	8	62
	♂	29	—	5	—	48	17	11	81
Totals	♀	150	—	36	16	176	88	46	277
	♂	116	—	31	—	227	86	48	341
Grand totals		266	—	67	16	403	174	94	618

(B) Vortex female by heterozygous male.

M417	♀	34	—	—	4	44	—	—	62
	♂	25	—	—	—	33	—	—	45
M418	♀	14	—	—	9	18	—	—	31
	♂	13	—	—	—	18	—	—	35
Grand totals		86	—	—	13	113	—	—	173

(C) *Dichaete vortex hairless female ex M389 by vortex male.*

M450	♀	54	2	18	23	—	22	—	36
	♂	56	—	27	—	9	23	—	60
Grand totals		110	2	45	23	9	45	—	96

THE ISOLATION OF AN ADDITIONAL VORTEX INTENSIFIER

During the course of all these later experiments a sharp outlook was kept for the occurrence of *dichaete vortex* males such as had been found in the first experiments with the mutant (table 1). In only one of the many cultures was such a male recorded (M446, table 7). This culture was likewise exceptional in the high number of *dichaete vortex* females

of the heterozygous type, over 40 percent of the dichaete females being vortex instead of under 20 percent.

Here was an opportunity to determine whether this male was of a different genetic constitution from ordinary dichaete males which do not show vortex. The male was out-crossed to a vortex female from stock. The absence of dichaete vortex males in the sons of the dichaete vortex male (M508, table 14) proves that he had not been a crossover; that is, that he was not homozygous for vortex III, for in that case practically all of his offspring should have been vortex.

The F_1 culture was exceptional in that there was a very high proportion of vortex among the dichaete females, just as had been the case in the parent culture 446. On the other hand none of the dichaete males showed vortex. So that it may be concluded provisionally that a modifying gene was present which was partially dominant among the females and not obviously dominant among the males. This difference is another expression of the already noted sex-limitation of the vortex character.

TABLE 14
Selection for dichaete vortex males.

No.	Parentage		D			
			D	v_o	v_o	+
M508	$v_o \text{ } \varnothing \times Dv_o \text{ } \delta$ ex 446	\varnothing	16	25	48	—
		δ	36	—	16	3
M550	$2Dv_o \text{ } \varnothing \times 2D \text{ } \delta$ ex 508	\varnothing	16	22	14	—
		δ	33	8	15	—
M608	$3Dv_o \text{ } \varnothing \times 3Dv_o \text{ } \delta$ 550	\varnothing	37	82	30	—
		δ	68	11	34	—

Culture 508 was so similar to 446 that it seemed probable that they were of the same constitution and that the single dichaete vortex male of 446 was a case of the dominance of the modifier, here effective even in the male. Another indication of this dominance is the suppression to a large extent of the changing over of vortex into wild-type in 446.

An F_2 culture was raised from two of the dichaete vortex females mated to two of the dichaete males from 508. While among the dichaete females the proportion of vortex was no higher than in F_1 , among the males 8 dichaete vortex males occurred in a total of only 41 dichaetes. These males are presumably to be looked upon as homozygous for the modifier. Three such males were crossed to dichaete vortex sisters and

the succeeding generation (M608) was characterized by the highest proportion of dichaete vortex yet observed. More than two-thirds of the dichaete females were vortex, indicating that more flies were homozygous for the modifier than in the previous cross. Another feature of these last two cultures was the absence of wild-type males in contrast to their usual occurrence in cultures free from the modifier. Thus the grade of vortex in all of its types has been raised to a high level by the action of the modifier, but this level is consistently higher in the female than in the male.

The gene for the modifier is known not to be in the third chromosome, unless very removed from dichaete, and the probabilities are that it is in the second chromosome.

The new experiments removed the suspicion that the inheritance of vortex depended upon some unusual chromosome condition. Thus, sterility did not appear in the new experiments, and its occurrence in the first experiments must have been a separate phenomenon. The locus of vortex III proved to be close to that of dichaete and not at the middle of the chromosome as required for "transposition". The other doubtful points have likewise fallen in line with a plural gene explanation.

THE MUTANT CHARACTER, FLIPPER

In culture 367 (table 5) a very small wild-type female was found and tests were made to determine whether she was genetically a dwarf or simply was exceptionally small because of some accident of development. In F_2 from a cross to vortex male no dwarfs reappeared, but a new mutant character appeared in the culture (M466). This new mutation resembled the sex-linked mutation club (see MORGAN and BRIDGES, Carnegie Publication No. 237 for figure). The whole fly was under-sized and was of shrunken appearance. The surface retained a wet appearance. The most obvious feature was the wing which remained in the folded condition in which they were when the fly emerged from the pupa case, and did not expand as wings normally do. These compact wings were held out and curved downwards like flippers.

When these flies began to appear it was observed that most of them were at the same time vortex. Counts were made which show that there was strong linkage between vortex and flipper (M466, table 15).

Several attempts were made to mate these flies together in order to obtain a stock of the mutation. All these matings failed except one, which gave 4 flipper females, 1 flipper male, and 1 wild-type male. Probably the wild-type male resulted from non-virginity of the mother.

TABLE 15
F₂ results from the cross of vortex flipper to wild.

	Wild-type	Vortex	Vortex flipper	Flipper
M466	190	17	32	3
M723	232	14	40	7
M724	229	19	25	5
Total	651	50	97	15

Because of the failure of all these matings the character flipper was lost, but it reappeared later in another culture in which vortex was used. In this case also quite extensive matings were made between vortex flipper females and their wild-type brothers. In one case only the mating produced a few wild-type offspring, from which two F_2 cultures were raised (723 and 724, table 15).

A calculation of the position of flipper was made on the basis of the three F_2 cultures of table 15. The flipper class (15) is a crossover class corresponding to the non-crossover vortex flipper class (97). Likewise the vortex class is a crossover class which corresponds to the compound wild-type class. The wild-type class is constituted from 3 non-crossover and 2 crossover classes ($3n + 2r$). The non-crossover class corresponding to the crossover class is calculated as 184 individuals. The total results give 65 crossovers to a total of 346 individuals, or 18.7 per cent of crossing over. The amount of crossing over between vortex and flipper is so large that it is improbable that the locus of flipper is to left of vortex since vortex is itself only 10 from the left end of the known chromosome. Flipper can be located approximately at a position 18.7 to the right of vortex or at 28.3 to the right of star.

SUMMARY

The foregoing experiments have shown that the character vortex is dependent upon or is modified by four mutant genes.

Of these genes the most essential one, without which the character is never known to have appeared, is situated in the second chromosome at a position 9.6 units to the right of star. However, this second-chromosome gene is by itself insufficient for the production of the vortex character.

The gene second in effectiveness is situated in the third chromosome very close to the locus of *dichaete* (11.7). This gene likewise is unable

to cause any development of the vortex character when acting alone. But in flies homozygous for vortex II, heterozygosity for vortex III enables about 20 percent of the heterozygous females to show the vortex character although no male of this constitution can show the character. Flies homozygous for both vortex III and vortex II are, if females, practically invariably vortex, while if males they are vortex except that toward the end of old cultures a small proportion of genetically vortex males of this homozygous type "change over" into wild-type. The usual stock of vortex is of this bigenic constitution.

During the experiments a dichaete stock was isolated in which a third gene was present which contributed to the development of the vortex character. In this stock a majority of the females homozygous for vortex II and heterozygous for vortex III showed the character, instead of only about 20 percent as in stocks in which this modifier is not present. A slightly greater percent of such females showed vortex when homozygous for the new modifier. In heterozygous condition this new modifier was almost without effect upon males of the heterozygous type, but in homozygous condition it made vortex show in a considerable proportion of the flies homozygous for vortex II and heterozygous for vortex III, while it eliminated the changing over of homozygous vortex II vortex III flies into wild-type flies. The locus of this modifier is probably in the second chromosome.

In the experiments involving streak a special relation between streak and the vortex was discovered. The vortex character was prevented from developing in streak flies even though such flies were homozygous for both vortex II and vortex III.

However, in the same experiments a special modifier was detected which to a considerable extent reversed this inhibition by streak. This modifier was a dominant situated in the second chromosome quite close to the locus of streak and probably to the right. The streak flies in which the vortex character appeared through the action of the modifier showed a type of vortex different from the usual one.

Throughout all of these experiments and in the various types of vortex a very striking fact was apparent, namely, that the grade of the vortex character and the proportion of flies showing that character was higher in the females than in males of the same genetic constitution.

INHERITANCE OF BRANCHING HABIT IN TOBACCO¹

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INTRODUCTION

In recent years a considerable number of papers have appeared on the inheritance of various quantitative characters in tobacco. Such characters are usually of the greatest economic interest although they do not lend themselves as readily to strict genetic interpretation as do qualitative characters, owing to the more pronounced influence of environmental conditions upon quantitative results. The general consensus of opinion among plant breeders now is, however, that quantitative characters behave according to Mendelian principles, even though the determination of the exact ratios may be impossible because of the multiplicity of factors concerned. Whatever the correct theory may be, the accumulation of facts from various sources and for various characters will no doubt strengthen the foundation for future theory and practice. Although a considerable number of characters in tobacco crosses have been studied by the writer since 1908, it is proposed to discuss here particularly the inheritance of branching or "suckering" habit, for the reason

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that this conspicuous character in tobacco seems to have received practically no attention by other investigators. It is, moreover, a character which possesses very considerable practical as well as scientific interest. The lateral branches or suckers which develop usually from the axils of the leaves grow to a considerable size and in most forms of tobacco culture must be broken off before harvest. This involves much labor, very often of a slow and tedious sort, as well as frequent mechanical injury to the leaves and possibly also a lowered yield since it is probable that the branches are partly instrumental in diverting foodstuffs from the commercial leaves to themselves. One and sometimes two or more removals of these lateral shoots or suckers are therefore necessary. It is safe to say that the ideal tobacco plant of the growers would possess no suckers. The removal of the terminal inflorescence together with the leaves immediately below (topping) is widely practised, and this naturally tends to stimulate to a considerable degree the rate of growth of the lateral shoots or suckers. These suckers are usually largest at the uppermost remaining node and gradually decrease in size to practically none below the fourth to sixth node. Varieties and also strains differ very much in this respect, however. The suckers may be present and of a considerable size not only at the node of every leaf, but they may also spring from the oldest nodes at the base of the stem where the leaves have long since perished in the normal physiology of the plant. We may therefore possess types with more suckers than remaining leaves.

The possibility that types of tobacco of limited- or non-suckering habit might be produced by simple selection or by crossing followed by selection, has occurred to many observers of tobacco culture. In the absence of any data on this character it would be quite unsafe even to venture a guess as to the practical results to be obtained in undertaking such a problem. As a result of the study of a cross between a small- and a large-suckering type of tobacco, it is believed that the evidence presented will be sufficient to place its behavior in inheritance in line with that of leaf number, size, and shape of leaves, as worked out by EAST and HAYES in particular.

It is admitted that the branching habit is not well adapted for study of inheritance as such, but if we are to understand various plant characters they must be dealt with as best we can. The branching habit is quite as readily modified by environmental conditions as yield of crop. The time of removal of the terminal bud, or the lodging of the plant are marked influencing factors, as well as the time of maturity of the

plants (itself not a definite character), in relation to time of suckering. These factors are to be considered as largely eliminated in the comparative data given, although some discrepancy may be due to time of maturity since the counts and weights were all taken at approximately the same time. The point is of commercial rather than biological significance, however.

REVIEW OF LITERATURE

It is not deemed necessary to consider in detail the literature upon inheritance in tobacco or of the inheritance of quantitative characters in general. Mention will be made of only a few investigations bearing most closely on the problem.

SHAMEL and COBEY (1907) under the general subject of tobacco breeding, consider briefly "the production of non-suckering types." They attach considerable importance to the variation in amount of suckering of different individuals in the same field but without adequate consideration of the part played by fluctuating variation in this regard in even the purest strains of seed. Judging, however, from the statement that strains were produced by two years' selection which were "almost free from suckers" in comparison with the ordinary seed which produced "many large suckers," the authors were apparently dealing with a strain of seed heterozygous for suckering habit. There are other conditions, however, which may account for their results, such as relative differences in actual time of maturity due either to germinal differences in this character or due indirectly to a variation in resistance to the Thielavia root-rot disease which in the writer's earlier experience in tobacco breeding was found to have almost completely vitiated results. The authors, in any case, lean towards the belief that environment, as such, produces permanent variations, permitting the fixing of type by selection, a theory which now has few followers.

The authors further add that a correlation exists between the number, shape, and character of the leaves borne by individual plants and the number and size of suckers produced by these plants. Many large suckers are said to be correlated with few, heavy, dark, and usually narrow, pointed leaves. This correlation is explained as being due to the large sucker branches taking from the plants the elements of plant food which would otherwise be utilized in the development of many broad, round leaves. No data for these conclusions are presented however.

The work of HAYES (1913) and of HAYES, EAST, and BEINHART

(1913) in Connecticut and Massachusetts, as presented in several papers, has carefully developed the facts of the inheritance of several characters in tobacco as far as they were obtainable, and with these data, laid the foundation of the generally accepted modern theory of the inheritance of quantitative characters according to Mendelian interpretation. The primary facts are the intermediate and uniform nature of the first filial generation in respect to the characters of the parents used in the cross, followed by distinct segregation giving a wide range of variability in the second generation; this range is usually as great or greater than the combined range of variability of the parents. The individuals selected in the F_2 generation may breed true to type, or may continue to show germinal variation. This has been shown to be true for various characters in tobacco, including leaf number, leaf area, length and breadth of leaves, and height of plants (excluding heterosis). This principle has also been shown to hold for various characters in several other plants by a considerable number of recent investigations.

The branching habit has received some attention from breeders of other plants, and although it may be that these habits are not in all cases similar to that in tobacco, they should perhaps be at least mentioned in this connection. Some of the cases are, however, merely brief statements of observations or based on limited data and cannot be satisfactorily correlated with the results presented in this paper.

SHULL (1908) concluded that in the case of the sunflower (*Helianthus annuus* var.) the branching habit followed Mendelian principles and that the branched habit was dominant to the unbranched.

SAUNDERS (1911) working with Stocks believes that the unbranched habit is recessive to the branched. The F_1 of branched with unbranched is branched and the pure unbranched reappears in the F_2 in what seems to be a proportion of 1 to 4, although this was apparently not satisfactorily determined. WEBBER (1912) reports on inheritance of branching habit in peppers. He finds the F_1 intermediate and gets marked variation in F_2 with the production of so-called giants and dwarfs, as a result of transferring fine and coarse branches. In connection with other studies, EMERSON and EAST (1913) report on the inheritance of number of stalks per plant in maize. "Stooling" in corn is probably quite different from branching, but on the other hand it does resemble it in many ways. This character was found to segregate upon crossing, in a manner similar to other quantitative characters.

NORTON (1915) has reported upon the inheritance of habit in the

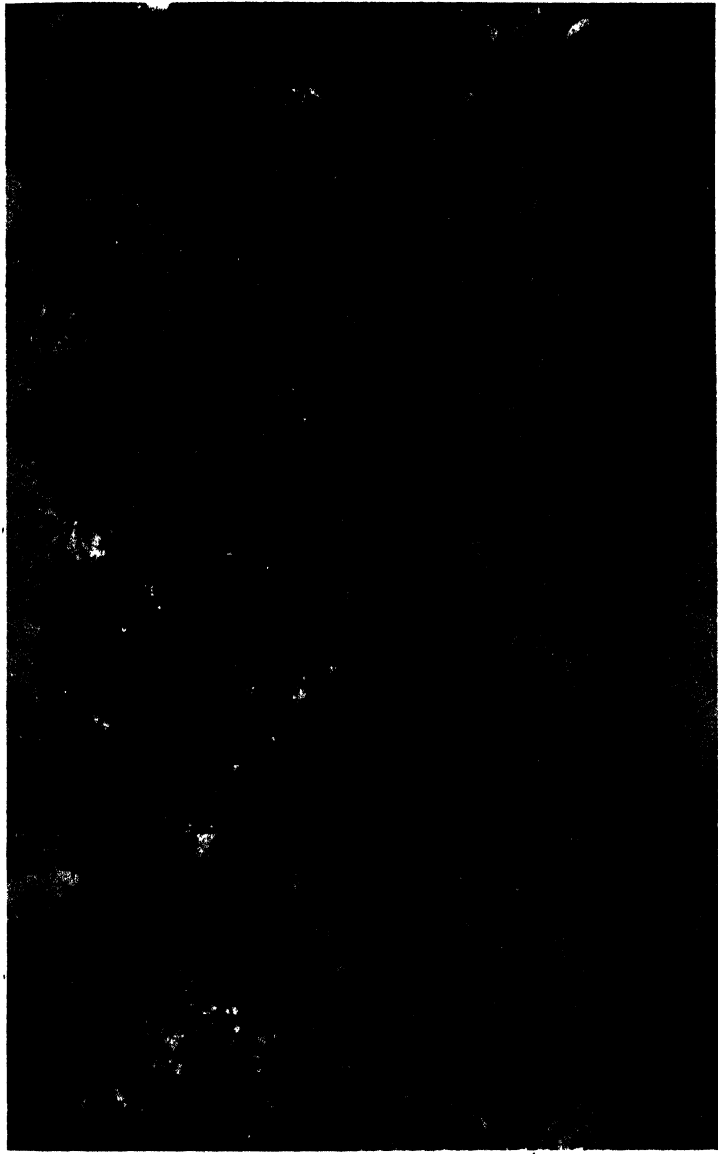
common bean in which the branching habit is at least characteristic for certain varieties. Nothing of a definite nature is stated in regard to this character unless it be considered that all bush beans are branched. He makes the statement, however, that in a cross between Refugee Wax and Blue Pod some lots homozygous for axial branching were isolated, many individuals of which showed signs of climbing.

METHODS USED

Studies on suckering habit are based particularly on reciprocal crosses of two types which in my cultures have gone under the names of Little Dutch and Cuban. The former is undoubtedly the same as that which has in the past been grown commercially in Ohio as filler tobacco. The leaves are relatively narrow and are distinctly erect (plate 3, P_1). The number of branches ranges from four to six and these are small in comparison with most varieties of tobacco. The number of suckers is much more constant than that of most varieties, which normally have a stronger tendency to sucker toward the base of the stalk. The Cuban type (plate 3, P_2) used is not the same strain as that now grown commercially under shade in the Connecticut valley, although it probably is one of the strains or types from which the shade-grown Cuban has been developed. It differs from the latter especially in that it does not grow quite as large and also in the fact that it branches much more profusely. The number of suckers is usually the same as the number of leaves, plus two to four additional large basal suckers which often grow to the height of the parent plant, beginning to develop soon after the plant is well established in the soil. That this type has bred true in its general conformation since it was first grown in connection with these studies (1908), has been repeatedly shown. The longevity of tobacco seed permits the growing of a considerable number of generations at any one time. In 1915 and again in 1916, succeeding generations of seed from the most vigorous plant in the cultures of the preceding years were grown side by side and certain measurements made of height, leaf number, and leaf size. The averages for these on twenty-five plants for the year 1915 are given in table 1, and show that no improvement or permanent change has occurred. This is in accord with the conclusions of HASSELBRING (1912) and of HAYES (1914) that modification through effects of environment of a southern type of tobacco grown in the north do not occur. Although the Little Dutch parent has not been subjected to a similar test in any one season, the data for the years 1908 to 1918 in-

TABLE I
Measurements of height, number, and size of leaves of inbred Cuban seed of different generations grown the same year. Averages of 25 plants.

Designation	Generation	Stalk	Leaves (inches)								Average breadth-index	
		Height in inches	Number	Top		Middle		Bottom		Average		
				Length	Width	Length	Width	Length	Width	Length		Width
I4 *	1	30.6	13.3	11.9	5.6	14.0	7.9	11.6	7.1	12.5	6.8	54.4
I40I	2	29.8	13.2	11.4	5.3	13.7	7.2	11.3	6.7	12.1	6.4	52.4
I40II	3	30.2	13.3	11.3	5.1	13.4	7.2	11.6	6.8	12.1	6.4	52.9
I40III	4	30.7	13.3	11.9	5.5	14.6	7.5	11.6	7.2	12.7	6.7	52.7
I40IIII	5	30.6	13.3	11.9	5.6	14.3	7.6	11.0	6.7	12.4	6.6	53.2



Progeny rows of strains isolated from the F_2 generation of the Little Dutch and Cuban cross showing the variation in suckering habit and other characteristics. (A) $42M$, (B) $42G21$, (C) $42F21$, (D) $42B11$, (E) $42A11$.

dicare that this variety is one of the most uniform strains of tobacco grown at the Experiment Station under all sorts of environmental conditions.

The usual precautions to prevent experimental error have been taken. Selfed seed has been obtained by covering the flower heads with manila paper bags. The seeds of individual plants were used in all cases, and these were sown in sterilized soils and the seedlings transplanted to separate rows in the field. The early experiments were complicated due to the stunting of the plants by the root-rot disease, but in recent years special care has been taken to avoid infested soils.

The parent types have been given numbers under which they were grown in the field, and later generations were merely designated by adding one digit to this number. The original Cuban seed was given the number 14, the next generation of seed plants 141, 142, up to 149 if desired. The following generation from 141 would then be 1411 or 1412, and so on. The cross of Little Dutch with Cuban, for the sake of convenience, was also given a number (42). The F_1 individuals selected for seed were then 421, 422, up to 429. The selection of individuals for growing the F_2 generations, however, were designated by letters, as 42A, 42B, 42C, and the F_3 selections became 42A1, 42B1, and so on.

The plants were topped as nearly as possible at the same relative point, which was just below the "bald sucker." The "bald sucker" is usually regarded as belonging to the terminal inflorescence. It is not believed that the results are altered by the fact that an arbitrary standard is chosen. This same objection has been used against the counts of leaf number of tobacco as a genetic measurement when the plants have been previously topped. As a check on this, a count was made of all nodes of the plants before topping and of leaf numbers after topping (tables 2 and 3). The last column in table 3 shows the ratio of leaf number to node number. This ratio, though showing some range, is believed to be sufficiently close to indicate a high correlation between the number of nodes per plant and number of leaves after topping. Since we have been dealing with plants apparently normal in growth, it is not believed that the facts brought out by ALLARD (1916) respecting the variable location of the "bald sucker" in stunted plants have played a part in these results.

In counting the number of suckers, only those which were large enough to warrant breaking off in commercial practice were included, that is, very small suckers which might later develop to a considerable size were

TABLE 2
Frequency distribution of number of leaves of *Little Dutch* and Cuban cross after topping.

Designation	Gen.	Classes for number of leaves per plant																								No.	Mean	
		6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26						
Little Dutch (15)																												
Cuban (14)																												
15 X 14	F ₁																											
14 X 15	F ₁																											
428	F ₂																											
424	F ₂																											
42V	F ₃																											
42M	F ₃																											
42N ₁	F ₄																											
42O ₁	F ₄																											
42X ₁	F ₄																											
42Y ₁	F ₄																											
42A ₁₁	F ₅																											
42B ₁₁	F ₅																											
42F ₂₁	F ₅																											
42G ₂₁	F ₅																											
42J ₁₁	F ₅																											

not included in the counts. Since every leaf axil or every node on the plant has the potential power of developing a lateral shoot, the line in any case would have to be drawn at some arbitrarily chosen point. The weights of the suckers in grams were taken immediately after their removal, on a torsion balance set conveniently in the field for this purpose. Linear measurements of plant height, and length and breadth of leaves were made with a steam-fitter's rule, which is especially convenient for this purpose.

EXPERIMENTAL RESULTS

In 1909 a very considerable number of varieties and strains of tobacco were crossed for the purpose of studying the inheritance of various characters as well as with the hope of developing new types suitable for commercial culture in Wisconsin. It is expected that the practical phase of the work will be discussed elsewhere. It may be mentioned, however, that a new type of tobacco has been produced by crossing two "strains" (believed to be mutations from the normal) of the Connecticut Havana variety. This new type (Connecticut Havana No. 38) has become quite popular in Wisconsin, considering the length of time it has been introduced.

Many of the types crossed in 1909 were quite similar in general habit and could not be relied upon for results of genetic interest, but where distinct varieties were crossed, "blending" in the F_1 generation was practically always observed. A large number of the F_1 generations from seemingly markedly different types when grown for the F_2 gave apparently no good indication of segregation. Whether this was due to lack of sufficient numbers of individuals or to various obscuring environmental factors is not clear, but the writer is tentatively, at least, inclined to agree with LOCK (1909) that the intermediate form of certain crosses in tobacco may persist in the second generation. This matter must, however, be left for further study and in the light of more recent investigations may be subject to analysis.

In 1913, however, it was noted especially that considerable variation was occurring in the F_2 of a cross of Little Dutch with Cuban and several of the more distinctive types were saved for seed to be grown as F_3 families the following year. The F_1 generation of this cross appeared strikingly intermediate in most respects (plate 3, F_1), especially as regards size, shape, and position of the leaf, and in number and size of suckers produced, although the latter habit was usually regarded as domi-

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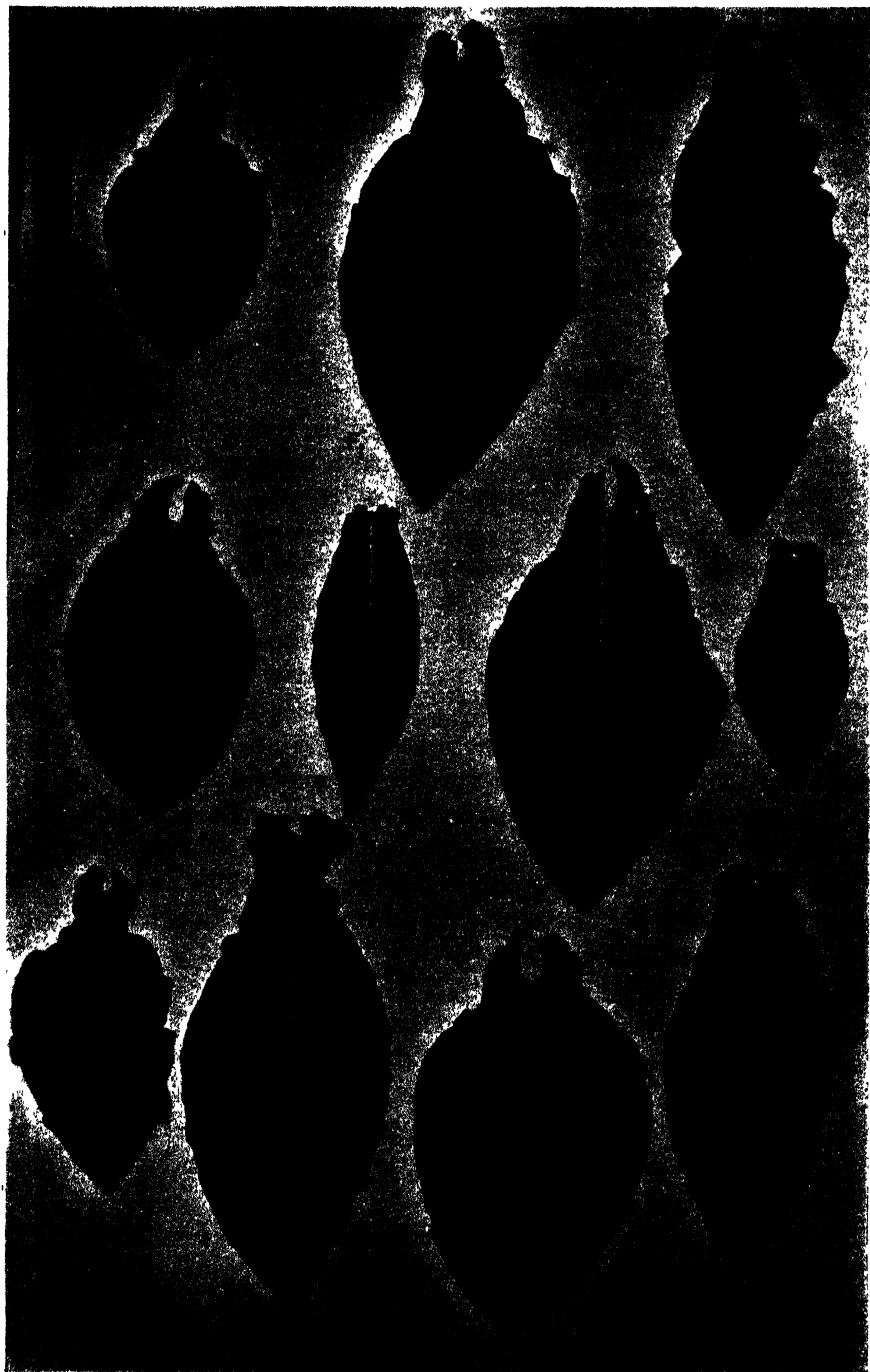
Designation							No.	Mean	Mean leaf number	Ratio of leaf number after topping to total node number
	37	38	39	40	41	42				
Little Dutch (15)							54	30.0	17.8	1 : 1.69
Cuban (14)							54	27.8	15.0	1 : 1.85
15 X 14							52	28.5	15.6	1 : 1.82
14 X 15							53	28.3	15.6	1 : 1.81
428	3	2	4				201	27.5	15.8	1 : 1.74
424							211	28.4	15.6	1 : 1.82
42V							53	27.9	16.2	1 : 1.72
42M	5	2	6	3	1	1	47	3.62	21.5	1 : 1.68
42N1	1						51	28.6	14.4	1 : 1.98
42O1							55	24.3	14.0	1 : 1.74
42X1							53	21.6	12.2	1 : 1.77
42Y1	1						54	28.9	15.6	1 : 1.85
42A11		1		1			52	31.9	19.5	1 : 1.64
42B11							52	22.4	11.9	1 : 1.88
42F21							55	29.0	16.3	1 : 1.77
42G21							50	19.5	9.4	1 : 2.07
42J11							50	21.5	11.6	1 : 1.85

nant to small suckers. It was also found that in crosses of Cuban with several other small-suckering types, as Connecticut Havana and Connecticut Broadleaf, the Cuban suckering habit apparently predominated. The Little Dutch and Cuban cross, however, was chosen as the best for a more detailed study of this character in tobacco. Twenty-four families from the F_2 generation as well as several from the F_3 have been grown; some were carried as far as the sixth generation. Marked segregation of leaf characters as well as of branching habit occurred (see plate 2).

Inheritance of number of suckers

The frequency distribution for number of suckers for 1917 in the Little Dutch and Cuban cross, together with the usual biometric terms, are given in table 4. It will be seen that the mean for number of suckers in the Little Dutch variety is $4.55 \pm .07$ as compared with $18.32 \pm .16$ for the Cuban type, a difference of about 13.7 suckers in the two types. The average number of suckers of the two parents for 1917 is 11.4, agreeing very closely with the mean found for the F_1 generation which is seen to be $11.28 \pm .22$ in one case, and $11.6 \pm .24$ in the reciprocal. The truly intermediate nature of the F_1 for number of suckers is therefore surprisingly close in this case. The standard deviation and coefficient of variability show, however, that the F_1 is somewhat more variable than the parents. It seems probable that this result may be due to other factors than that of germinal variation, namely, to heterosis. Turning to the F_2 generations (reciprocals) for this year, it may be seen by the frequency tables that the range of variation is practically equal to that of both parents combined. The mean for the reciprocals will be noted to be very close to that of the F_1 , a fact which seems to be of some significance in indicating the dominating feature of the suckering character in the second generation. The standard deviation shows that the F_2 varies considerably more from the mean than does either of the parents or the first generation. The standard deviation for the Little Dutch is only $.75 \pm .05$, and of the Cuban $1.64 \pm .11$, while that of the F_1 is $2.26 \pm .15$ as compared with $4.07 \pm .14$ for the F_2 .

The F_3 and succeeding generations, show in a more striking manner the marked segregation which has occurred in this cross. Families have been produced which range in means from $4.11 \pm .09$ suckers (42J11) to a family with a mean of $15.40 \pm .33$ suckers (42V). Furthermore, the standard deviations show that families like 42J11 and 42A11 are



LEGEND FOR PLATE 2

Illustrating the variation in leaf size and leaf shape produced in the F_2 of the Little Dutch and Cuban cross. All leaves selected from the same relative position on the plant. P_1 , Cuban parent; P_2 , Little Dutch parent. F_1 , first generation. A, B, F, G, G2, O, S, and X are from strains isolated from F_2 and represent typical leaves of families 42A, 42B, etc., respectively.

breeding relatively true, having deviations of only $.98 \pm .065$ and $1.22 \pm .08$, respectively. Other families, however, as 42G2I and 42M show a standard deviation as large or larger than the F_2 generation, being $4.03 \pm .27$ and $4.81 \pm .23$ respectively (plates 6, 7 and 8).

It may be objected that these figures do not represent in an entirely correct way the true state of affairs, in that they do not take into consideration the relative time of maturity of the two families. If we let the time of the opening of the first flower of the plant represent the relative time of maturity (table 5), we find that 42G2I (heterozygous for this as well as for other factors) is a relatively early type, consequently, the production of numerous suckers is favored, and that 42M is a relatively late type, consequently not so much disposed to the production of numerous suckers within the given time. The means, therefore, would be lower for the one and higher for the other. These cases are extremes, however, and serve only to show that the problem may be complicated

TABLE 5

Frequency distribution of time of flowering of Little Dutch and Cuban cross, 1917.

Designation	Gen.	Class centers in days for time of flowering																No.	Mean
		2	5	8	11	14	17	20	23	26	29	32	35	38	41	44			
Little Dutch (15)	P ₁								17	9	15	7	5				53	27.5	
Cuban (14)	P ₁						1	11	24	13	1	2	2				54	23.8	
15 × 14	F ₁					11	24	9	6	2							52	20.9	
14 × 15	F ₁					15	20	11	6	2							54	20.7	
428	F ₂				2	17	31	25	30	31	27	27	7	4	1		202	27.0	
424	F ₂					17	18	46	43	35	18	23	6	3	1		210	26.7	
42V	F ₂								12	9	17	6	5	3			52	28.5	
42M	F ₂									4	11	4	12	8	2		41	33.1	
42N1	F ₄					16	9	15	5	2	1	2					50	21.7	
42O1	F ₄					6	18	11	10	1	1	2					49	22.5	
42X1	F ₄				9	7	24	4	3	2	1	2					52	17.2	
42Y1	F ₄					3	4	12	12	8	5	4	4	2			54	27.3	
42A11	F ₅						1		3	6	11	12	14	4	1		52	31.2	
42B11	F ₅				1	5	26	13	5	2	1						53	18.4	
42F21	F ₅							2	13	23	12	4	1				55	26.3	
42G21	F ₅	5	7		17	5	12	2	1	2		1					52	12.5	
42J11	F ₅					2	24	18	2	4	1		1				52	19.4	
Actual dates of flowering		July																	
		14	17	20	23	26	29	1	4	7	10	13	16	19	22	25			
		16	19	22	25	28	31	3	6	9	12	15	18	21	24	27			

s, 1917.

Designation	Ge	Mean	Standard deviation	Coefficient of variation
Little Dutch (15)		4.55 \pm .07	.75 \pm .05	16.43 \pm 1.21
Cuban (14)		18.32 \pm .16	1.64 \pm .11	8.95 \pm .61
15 \times 14		11.28 \pm .22	2.26 \pm .15	20.08 \pm 1.41
14 \times 15		11.60 \pm .24	2.49 \pm .17	21.52 \pm 1.51
428		11.33 \pm .20	4.07 \pm .14	35.90 \pm 1.38
424		10.27 \pm .20	4.11 \pm .12	40.00 \pm 1.56
42V		15.40 \pm .33	3.49 \pm .23	22.68 \pm 1.50
42M		13.90 \pm .51	4.81 \pm .36	34.64 \pm 2.89
42O1		11.08 \pm .23	2.07 \pm .16	18.68 \pm 1.35
42N1		11.69 \pm .29	2.87 \pm .20	24.57 \pm 1.82
42X1		9.74 \pm .26	2.76 \pm .18	28.42 \pm 2.06
42Y1		8.29 \pm .26	2.64 \pm .183	31.81 \pm 2.42
42A11		5.23 \pm .12	1.22 \pm .08	23.30 \pm 1.69
42B11		8.46 \pm .23	2.44 \pm .16	28.80 \pm 2.05
42F21		9.22 \pm .37	2.94 \pm .20	31.88 \pm 2.35
42G21		10.62 \pm .38	4.03 \pm .27	38.02 \pm 2.90
42J11		4.11 \pm .09	.98 \pm .06	23.80 \pm 2.06



Typical plants of Little Dutch parent (P_1) first generation of cross between Little Dutch and Cuban (F_1) and of Cuban parent (P_2). "Topping" stage illustrating general growth habit.



Second generation individuals of cross between Little Dutch and Cuban. (A) 42M, (B) 42G, (C) 42F. "Topping" stage of growth.



Second generation individuals of cross between Little Dutch and Cuban. (D) $42E$, (E) $42H$, (F) $42L$. "Topping" stage of growth.

by the factor of maturity. In the majority of cases of the families studied, this factor cannot be said to have played a part.

The strain 42G the original plant of which is shown in plate 4, B, has been in many respects, the most interesting of all the families studied. It appeared first in 1914 and was at once recognized as a dwarf form. This plant was only 17 inches high and possessed twelve small leaves and fifteen suckers. Although the strain has now run to the sixth generation, it shows no sign of giving off any homozygous forms, although several distinct types of plants have occurred in greater or less numbers. In 1917 a number of deformed plants or monstrosities appeared. Certain of the top leaves appeared to make no growth on one side, hence resulting in a curling to one side. The first flowers appeared very early and were malformed, various degrees of catacorolla and fasciation appearing which terminated the inflorescence, though the plants soon produced branches which flowered normally, but whose flowers were much smaller than those on the normal plants.

Family 42F (plate 4, C) on the other hand seemingly bred true in all characteristics from the start. This plant was selected in the F_2 on account of its desirable habit of growth and because it appeared to possess commercial possibilities. No variation which could be taken as germinal has been observed up through the F_5 generation.

The results in 1918 for number of suckers (table 6) are quite in accord with the results of 1917. The mean number of suckers for the Cuban type is 19.14 ± 1.70 , while that of the Little Dutch is $4.14 \pm .05$. The mean of the F_1 is $15.69 \pm .02$, considerably above the average of the two parents, which is 11.63. The explanation of this fact in view of the results in 1917, where the average of the parents and the obtained mean for F_1 were very close, probably lies in a more favorable condition for heterosis (the stimulus of heterozygosis) to occur. The F_1 generation is again somewhat more variable than the parents, as shown by the standard deviation, but only slightly more so than the Cuban. The standard deviation for the F_1 is $2.09 \pm .01$ as compared with $1.86 \pm .12$ for the Cuban parent and only $.57 \pm .07$ for the Little Dutch parent.

The F_2 , however, shows a standard deviation of $4.54 \pm .08$ and a mean of $11.98 \pm .11$, which again shows the strong tendency on the part of the majority of the F_2 population toward the suckering habit of the Cuban parent.

In 1918 the succeeding generations of some of the F_3 families were grown side by side up to the F_6 , together with the parents, F_1 and F_2 ,

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Designation	Gen.	No.	Mean	Standard deviation	Coefficient of variation
Little Dutch (15)	P ₁	58	4.14 ± .05	.57 ± .07	13.79 ± .88
Cuban (14)	P ₁	54	19.14 ± 1.70	1.86 ± .12	9.71 ± .63
14 × 15	F ₁	55	15.69 ± .02	2.09 ± .01	13.35 ± .87
424	F ₂	118	11.98 ± .11	4.54 ± .08	37.90 ± .76
42A	F ₂	44	8.61 ± .32	3.13 ± .24	36.38 ± 2.92
42A _I	F ₂	43	12.30 ± .29	2.88 ± .21	23.48 ± 1.79
42A _{II}	F ₂	56	5.26 ± .09	.97 ± .06	18.45 ± 1.48
42A _{III}	F ₂	55	6.45 ± .20	2.26 ± .14	35.05 ± 2.51
42J	F ₂	59	6.81 ± .19	2.18 ± .13	32.05 ± 2.18
42J _I	F ₂	57	5.47 ± .15	1.71 ± .11	31.37 ± 3.41
42J _{II}	F ₂	57	5.01 ± .10	1.16 ± .07	23.14 ± 1.68
42J _{III}	F ₂	57	4.77 ± .11	1.25 ± .08	26.25 ± 1.76
42G	F ₂	57	17.14 ± .03	2.93 ± .18	17.14 ± 1.75
42G ₂	F ₂	60	16.70 ± .36	4.19 ± .26	25.13 ± 1.64
42G _{2I}	F ₂	57	14.85 ± .04	4.04 ± .25	27.21 ± 1.83
42M	F ₂	44	15.63 ± .35	3.48 ± .25	22.28 ± 1.67
42M _I	F ₂	47	15.74 ± .44	4.49 ± .31	28.56 ± 2.14
42V	F ₂	49	15.28 ± .28	2.95 ± .20	19.32 ± 1.36
42V _I	F ₂	52	13.12 ± .23	2.37 ± .16	18.07 ± 1.24
42P	F ₂	45	13.04 ± .43	4.33 ± .31	33.18 ± 2.60
42R	F ₂	48	5.41 ± .17	1.76 ± .12	32.56 ± 2.47
42T	F ₂	59	14.22 ± .34	3.92 ± .24	27.54 ± 1.82

this being possible owing to the long period of viability of tobacco seed. The conditions were consequently good for the comparison of the different generations. In family 42A it may be noted that the mean has shifted upward and downward. This is better shown by the standard deviation which gradually shifted downward to a point approaching the homozygous condition but which rises again in the F_6 generation. In family 42J, however, the mean has been gradually shifted downward as has also the variability as shown by the standard deviation. Whether this could be still further lowered is in doubt. The results in the F_4 , F_5 , and F_6 are, however, too close to warrant much significance being attached to them in this respect, and it seems quite probable that this family has been practically homozygous for number of suckers since the F_4 generation. Other families, as 42G, as indicated by the standard deviation, have shown no tendency to decrease in variability thus far, and are almost as variable as the F_2 .

The coefficients of variability are not regarded as a good measure of the conditions in these studies and the comparison of the different generations can be much better supplied by growing them in the same season and comparing the standard deviations, than by comparing the coefficient of variability under dissimilar conditions.

Inheritance of weight of suckers

It will be shown that considerable correlation exists between weight of suckers and number of suckers. In a study of suckering habit, however, it is just as necessary to consider size of the suckers as their number and it is not to be expected that those families which show the greatest number of suckers will necessarily show the greatest weight. A plant may form many small suckers or relatively few large suckers within a given period (plate 7, A and C).

The first data on weight of suckers were taken in 1914. The data for 1915 and 1917 only, will be presented, however, though the results of the five years bear out the conclusions drawn. Table 7 for sucker weight in 1915 shows the mean weight of the Little Dutch variety to be 22.2 ± 1.08 decigrams as compared with 49.63 ± 1.14 for the Cuban. Owing to the fact that the weights were taken relatively late, the Little Dutch suckers show a weight greater than normal as compared with Cuban, i.e., the Cuban suckers had reached their maximum weight some time before the weights were recorded, permitting the Little Dutch to make a proportionate increase. The mean weight of the F_1 closely approaches that

TABLE 7
Frequency distribution of weight of suckers per plant in cross between Little Dutch and Cuban, 1915.

Designation	Gen.	Class centers in decigrams for weight of suckers															No.	Mean	Standard deviation	Coefficient of variation					
		5	15	25	35	45	55	65	75	85	95	105	115												
Little Cuban (15) Cuban (14) 15 X 14 423 42A 42B 42C 42D 42E 42F 42G 42H 42I 42J 42K 42L	P ₁	6	20	9	12	3												50	22.20 ± 1.08	11.32 ± .76	50.99 ± 3.43				
	P ₁			4	10	16	12	7	4									54	49.62 ± 1.14	12.42 ± .80	25.09 ± 1.55				
	F ₁			4	4	24	28	18	8	7	6	1						100	49.00 ± 1.15	17.15 ± .81	35.00 ± 1.66				
	F ₂	52	145	157	148	67	56	15	10	5	5	2	1					666	31.20 ± .47	18.12 ± .33	58.10 ± 1.07				
	F ₃	7	5	15	14	5	1	2										50	37.80 ± 1.86	19.55 ± 1.31	51.71 ± 3.48				
	F ₃	2	5	10	12	7	2	2	3	1								44	46.81 ± 1.89	18.62 ± 1.33	39.76 ± 2.85				
	F ₃	8	14	10	5	3	2	2	1									45	25.02 ± 3.51	17.51 ± 1.24	70.06 ± 4.98				
	F ₃	1	5	7	11	10	7	4	1	1	1							48	41.45 ± 1.81	18.65 ± 1.28	44.99 ± 3.09				
	F ₃			15	13	9	7	2	2									49	40.71 ± 1.46	15.17 ± 1.03	37.25 ± 2.53				
	F ₃	15	17	9	6	3												50	18.00 ± 1.13	11.87 ± .80	65.96 ± 4.38				
	F ₃	4	7	13	10	11		4	1									50	32.60 ± 1.57	16.56 ± 1.11	50.76 ± 3.42				
	F ₃	1	3	11	13	8	8	4	2	1								51	40.68 ± 1.61	17.06 ± 1.13	41.95 ± 2.80				
	F ₃	8	15	15	4	6	1											49	22.55 ± 1.23	12.86 ± .87	57.03 ± 3.88				
	F ₃	1	4	11	19	10	2	2		1								50	35.60 ± 1.32	13.91 ± .93	39.08 ± 2.02				
F ₃		1	5	15	12	5	6	3	1	2							50	47.40 ± 2.10	22.09 ± 1.49	46.61 ± 2.94					
F ₃		3	19	16	6	4	2										50	24.00 ± 1.13	11.87 ± .80	49.41 ± 3.33					



Typical plants of Little Dutch parent (P_1), first generation of cross between Little Dutch and Cuban (F_1), and of Cuban parent (P_2) illustrating "suckering" habit.



Second generation individuals of cross between Little Dutch and Cuban, illustrating segregation in "suckering" habit. (A) $42V$, (B) $42G$, (C) $42M$.



Second generation individuals of cross between Little Dutch and Cuban, illustrating segregation in "suckering" habit. (D) 42F (E) 42J, (F) 42B.

of the Cuban parent, a fact which is again believed to be due to the stimulus of heterozygosis. The most significant feature in the results for 1915 are that, although a range of variation greater than that of the parents combined occurred in the F_2 , the F_3 generations show a distinct segregation into families some of which, as 42F, show a smaller-suckering habit than the Little Dutch parent, while others like 42K closely approach the Cuban parent in mean weight of suckers.

Passing to the results of 1917 (table 8), which illustrate in a more satisfactory way the important points, we find that the mean weight of the Little Dutch suckers is $9.4 \pm .62$ as compared with 46.4 ± 1.41 for the Cuban. The mean of the F_1 and its reciprocal are very close and compare quite favorably with the average of the two parents, showing, however, some heterosis. The mean weight for the F_2 and its reciprocal is on the average about midway between that of the two parents, and the F_3 families show means approaching that of the small-suckering parent to above that of the large-suckering parent. Considering the standard deviation of the weights of suckers for 1917, it should be first noted that the Little Dutch is not nearly as variable as the Cuban variety, and if we consider the average of the two as a measure of the standard deviation of the parents, we find the F_1 is only slightly more variable than the average of the parents as regards suckering habit and not as variable as the Cuban parent considered alone. The F_2 shows some increase in variability, but not as much as expected, although the range is equal to that of both parents combined.

Strain 42AII has a comparatively low standard deviation ($8.35 \pm .58$) but is probably not homozygous since results in 1918 for number of suckers apparently showed increased variation in the F_3 . Strain 42BII, 42V and 42M have greater deviation from the mean than the F_2 and the two former are apparently considerably more heterozygous for weight than for number of suckers. It is worthy of note that whereas 42JII showed the least deviation from the mean in number of suckers 42AII showed the lowest deviation in weight of suckers, but the 42M, which showed the greatest deviation as regards number of suckers, also gave the greatest variation in weight of suckers. The question therefore arises as to the relation existing between the number and weight of suckers per plant. It will be shown that a significant correlation exists between these characters. The inheritance of weights of suckers in the cross of Little Dutch and Cuban is therefore similar to that of number of suckers. The marked variation in size of suckers as well as in number of suckers in this cross is illustrated in plates 6, 7 and 8.

TABLE 8
Frequency distribution of weight of suckers of Little Dutch and Cuban cross, 1917.

Designation	Generation	Class centers in decigrams for weight of suckers												No.	Mean	Standard deviation	Coefficient of variation
		5	15	25	35	45	55	65	75	85	95						
Little Dutch (15) Cuban (14)	P ₁	28	14	3									45	9.4 ± .62	6.19 ± .44	65.85 ± 4.68	
	P ₁		1	4	13	13	10	4	3	1			49	46.4 ± 1.41	14.66 ± .99	31.60 ± 2.15	
15 × 14 14 × 15	F ₁	1	7	17	15	7	1	1					49	30.5 ± 1.09	11.40 ± .77	37.38 ± 2.54	
	F ₁	3	8	14	16	8	1	0	1				51	30.1 ± 1.25	13.26 ± .88	44.07 ± 2.94	
428 424	F ₂	41	46	32	31	24	10	3	1	1			189	25.2 ± .82	16.82 ± .63	66.75 ± 2.31	
	F ₂	31	46	52	33	22	3	6	0	1			194	30.9 ± .67	14.00 ± .48	45.30 ± 1.55	
42V 42M	F ₃	5	6	13	9	8	8	1	2				52	34.0 ± 1.65	17.66 ± 1.17	51.95 ± 3.43	
	F ₃	11	10	9	1	6	3	0	1				41	23.7 ± 1.87	17.80 ± 1.32	75.12 ± 5.59	
42N1 42O1 42X1 42Y1	F ₄	3	10	21	11	1	0	1					47	27.3 ± 1.02	10.34 ± .71	37.90 ± 2.63	
	F ₄	2	4	17	12	5	3	2					45	31.8 ± 1.35	13.45 ± .95	42.30 ± 3.00	
	F ₄	3	3	12	19	7	4	2					50	33.8 ± 1.28	13.49 ± .91	39.91 ± 2.69	
	F ₄	11	13	17	2	2	1	0	1				47	20.6 ± 1.33	13.56 ± .94	65.84 ± 4.57	
42A11 42B11 42F21 42G21 42J11	F ₅	16	20	9	2								47	14.3 ± .82	8.35 ± .58	58.16 ± 4.40	
	F ₅	1	6	11	15	5	6	6	1	1			52	38.4 ± 1.65	17.64 ± 1.16	45.85 ± 3.03	
	F ₅	4	15	21	7	3							50	23.0 ± .93	9.79 ± .66	41.73 ± 2.81	
	F ₅	3	3	5	18	12	5	1	1	2			50	48.8 ± 1.61	16.94 ± 1.14	34.71 ± 2.34	
	F ₅	1	12	17	9	10	1	1					51	39.3 ± 1.18	12.53 ± .83	31.88 ± 2.09	

Correlations

As already stated, a marked correlation appears to exist between the number and weight of suckers per plant in tobacco. Table 9 shows the distribution and correlation of these characters in 374 plants of the F_2

TABLE 9
*Correlation between number of suckers and weight
of suckers in Little Dutch \times Cuban F_2 .*

		Class centers of No. of suckers							Totals
		3	6	9	12	15	18	21	
Class centers of weight of suckers	50	13	26	16	7	3			65
	150	3	18	31	20	16	7		95
	250		15	16	20	15	10		76
	350		10	11	18	16	12	1	68
	450		6	6	16	10	6	1	45
	550			1	4	6	1		12
	650			1	3	4	2		10
	750						1		1
	850			1	1				2
Totals		16	75	83	89	70	39	2	374

Coefficient of correlation, $.423 \pm .028$

generation of Little Dutch and Cuban grown in 1917. The number and weight of the suckers of each plant were recorded as they were broken off. The coefficient of correlation is found to be $.423 \pm .028$ indicating a relatively good correlation between number and weight of suckers. It is quite likely that a greater correlation would have been obtained if a pure strain of seed had been used. This would not have brought out the point which it is desired to emphasize since the variability in such a case would be merely due to fluctuating variation.

In the same manner, it is shown (table 10) that no correlation between number of leaves and number of suckers per plant can be said to exist, the coefficient obtained being $-.012 \pm .05$. According to SHAMEL and COBEY (1907) a significant negative correlation should be obtained, i.e., a large number of leaves should result in fewer suckers, since such leaves make it physiologically more difficult for suckers to grow. While admitting that such a physiological relationship may exist, the results presented here indicate that there is little or no genetical relationship between these characters.

With respect to the relation of the shape and size of the leaf and size of suckers, it is to be expected that some negative correlation may exist,

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h and Cuban cross.

Designation				Suckers	
	Average		Breadth- index	Mean number	Mean weight (grams)
	gth	Width			
14 Cuban	3	7.1	57.7	18.3	464
15 Little Dutch	7	7.9	40.1	4.5	94
14 X 15	8	7.9	47.0	11.6	30*
15 X 14	2	8.0	46.5	11.3	305
424	3	7.2	44.1	10.3	309
428	9	7.8	49.0	11.3	252
42A11	1	9.2	65.2	5.2	143
42B11	9	6.2	41.6	8.5	385
42F21	1	9.8	57.3	9.2	230
42G21	5	3.4	32.3	10.6	485
42J11	2	9.6	63.1	4.1	393
42N1	4	7.5	40.7	11.7	273
42O1	2	7.7	40.1	11.0	318
42X1	1	4.6	30.4	9.7	338
42Y1	6	10.4	66.6	8.3	206
42M	7	8.2	52.2	13.9	237
42V	3	10.9	71.2	15.4	340

TABLE 10

Correlation between number of leaves and number of suckers per plant of Little Dutch \times Cuban F_2 .

	Number of suckers per plant																	Totals
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
Number of leaves per plant																		
11		1		1						1	1							4
12							1	1		1		1						4
13			4			1			2	4	1	7	1			1		21
14				3	3	3	1	3	2	1	2	4	2	3	3	1		31
15	1		1	2	3	3	1	1		2		3	3	2	2	1		25
16				3	2		1	1		5	1	1		2		3		19
17			1	1		3	2	2	3	2			1	2		2		19
18	1	1	1					2	1	2		1	1			1		11
19					3	1	2						1		2	2	2	13
20		1	1		2	1	1						2	2				10
			1															1
					1													1
Totals	2	3	9	10	14	12	9	10	8	18	5	17	11	11	7	11	2	159

Coefficient of correlation, $-.012 \pm .05$

since a large-leaved plant may tend to shade the suckers in some degree, hence reducing their rate of growth. It is not expected that this point can be cleared up definitely with the data at hand, but it has been thought best to present all the data for the various families in 1917 in the shape of a table of averages (table 11) of leaf measurements rather than to construct a correlation table. The breadth-index is given as a measure of leaf shape. It is obtained by dividing the breadth by the length and expressing the result in percent. No measurements have been made on leaf area, but a good idea of leaf size may be obtained by consulting the average length and breadth of the top, middle, and bottom leaves. The relative size and shape of leaves in this cross are illustrated in plate 2, showing that material for such a study was at hand. Without going into a comparison of these data in detail, suffice it to say that the family with the lowest breadth-index (42X1) does not possess the greatest average number of suckers. In the same way the family with the largest breadth-index (42V) does not possess the lowest average number of suckers (in fact it has the greatest number) nor the smallest average weight of suckers. Again the family with the largest-sized leaves does not possess the smallest number or weight of suckers. There seems to be no indication in these data, therefore, that shape and size of leaves are consistently associated in any way with number or weight of suckers.

DISCUSSION OF RESULTS

The Little Dutch and Cuban cross in tobacco offers a striking case of combination of parental characteristics and uniformity of type in the F_1 generation, and of segregation in F_2 into a great variety of forms in regard to various morphological characters. Furthermore, some of these F_2 types may breed true from the start with respect to certain characters; other types become fixed in the fourth or succeeding generations; and still others apparently continue in a heterozygous condition through the sixth generation.

Although ratios in these various characters have been sought for, nothing satisfactory has been obtained which could be in any way made to conform to typical Mendelian ratios with any number of genes considered. This is not believed, however, to signify that the characters are not conforming to Mendelian principles. The assumption of "multiple" factors and continuous variation as propounded by NILSSON-EHLE (1908, 1909) and EAST (1910) and later by a considerable number of investigators for various characters, can be made to explain the facts as they occur. This theory assumes that the parent plants differ in two or more separate genes for the character in question. These independent genes, allelomorphic to their own absence, are capable of adding to the character and the heterozygous condition of any unit is half of the homozygous condition. Little can be gained at present by passing into a discussion of the number of probable genes involved in the inheritance of the suckering habit. There are undoubtedly a great number of such factors concerned, especially in weight. In any case the same mathematical scheme as applied by HAYES (1913) for number of leaves in tobacco or by various other writers for quantitative characters might be developed to fit the situation at hand. On the other hand, as assumed by SHULL (1914), it is probable that some of the variability in the F_2 and succeeding generations may be due to heterosis.

If we can assume that the small-suckering habit of the Little Dutch is an expression of an unbranched condition and the large-suckering habit of the Cuban is the branched condition, the question arises as to which is the recessive condition. There seems to be little doubt that if we can justifiably use this term in this case, the unbranched or small-suckering habit tends to be recessive to the large-suckering habit. This conclusion is based largely on the predominance of large-and-numerous-suckering types in the F_2 and on the fact that several small-suckering F_2 individuals have shown a tendency to breed true, whereas the large-suck-

ering types have been more difficult to obtain in a homozygous condition. This is in accord with Miss SAUNDERS's observations on the inheritance of branching habit in stocks. Miss SAUNDERS (1911) was apparently dealing with a true unbranched parent, rendering her results perhaps more easy of interpretation.

Our results with tobacco are more similar possibly to the data of EMERSON and EAST (1913) on number of stalks per plant in corn. The results are at least closely comparable, as are also those of WEBBER (1912) in a study of branching habit in peppers.

The origin and behavior of family 42G in this cross has been very interesting. The original plant and a certain proportion of its offspring are truly "dwarfs" (figure 1, B), if such a word can be used with any degree of definiteness. Since the dwarf has not yet been obtained in the homozygous condition, considerable variation in height occurs, as may be seen by referring to family 42G21 in table 12. The true dwarfs, however, lie considerably below the mean given.

The behavior of 42G has been in many respects similar to that described by STOUT (1915) for dwarf plants in *Hibiscus oculirosus*. Its origin, however, is clearly that of a variate due to crossing two distinct varieties of tobacco belonging to the same species. No one would recognize this plant as related in any way to its grandparents. Furthermore, no plant in any way resembling it has appeared in several thousand F_2 plants grown. The similarity of its behavior to mutating forms and



FIGURE 1.—Strains 42M and 42G growing side by side showing the characteristic of a "giant" (A) and the "dwarf" (B) forms produced in the Little Dutch and Cuban cross.

TABLE 12
Frequency distribution of height in Little Dutch and Cuban cross, 1917.

Designation	Gen.	Class centers for height of plants in inches												No.	Mean
		5.5	11.5	17.5	23.5	29.5	35.5	41.5	47.5	53.5	59.5	65.5	71.5	77.5	83.5
Little Dutch (15)	P ₁							17	30	3				50	45.8
Cuban (14)	P ₁								1	22	24	3		50	56.9
15 X 14	F ₁								3	20	26			49	56.3
14 X 15	F ₁								3	33	15			51	54.9
428	F ₂						18	50	60	44	16	3		191	53.9
424	F ₂				1	9	40	65	60	19	1			195	54.7
42V	F ₂					3	9	21	14	2				49	53.8
42M	F ₂								4	17	14	6	1	42	69.0
42N1	F ₄					2	7	20	19					38	53.1
42O1	F ₄					3	22	18	5					48	50.6
42X1	F ₄				2	20	11							53	39.8
42G1	F ₄					2	5	16	20	2				45	56.7
42A11	F ₅					2	15	18	6					41	51.5
42B11	F ₅					3	21	26	2					52	44.6
42F21	F ₅							17	29	9				55	52.6
42G21	F ₅	4	7	3	11	11	9	5	2					52	26.1
42J11	F ₅					12	34	5						51	40.6

especially to mutating dwarfs is sufficiently striking to warrant mention. The author is inclined to believe, however, that it is a case of "true" segregation.

Another abnormality which has arisen in this cross, which has not occurred in either the Little Dutch or Cuban parent or in any other known type of tobacco, was first noted in 1917. This abnormality has evidenced itself as a peculiar "physiological weakness" of the plants occurring late in the season. The veins of the leaves starting from below first show signs of browning and deterioration (not decay) (figure

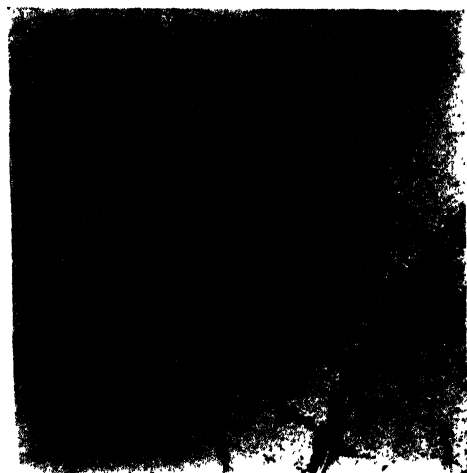


FIGURE 2.—Small portion of area of leaf of 42A showing the characteristic pattern on leaves of plants exhibiting "physiological weakness".

2). This results in an intermediate stage in a peculiar netting of the leaves, which finally dry up from the bottom. In 1918 it was noted that the suckers coming from the base of plants cut off a few weeks previously began to die from this trouble indicating strongly that the difficulty arises at the roots of the plants, which apparently lose their power to function properly, although close examination showed no striking abnormality of these organs. In the F_2 these plants occurred in a ratio of about 1 to 25. Two F_3 strains, 42A and 42M, were uniformly affected in 1917 and were again affected in 1918 in a similar manner. This characteristic was therefore behaving as a recessive. The appearance of this variation, entirely new and different from anything observed in a large number of cultures grown under a wide variety of

conditions for a number of years, is sufficiently significant to warrant mention, since it indicates the production of an entirely new character in tobacco so far as my observations have gone.

SUMMARY

The inheritance of the branching or suckering habit of tobacco has been studied in a cross between a Cuban type possessing many large suckers and a Little Dutch strain possessing few and small suckers. The conclusions are based largely on counts and weights of suckers of the parental types and the succeeding generations of the cross made over a period of several years under widely varying environmental conditions. The conclusions are more specifically drawn, however, upon the data here presented in which the parents and several succeeding generations of the cross were grown in the same year under identical conditions. Some data are also given on correlations between number of leaves and number and weight of suckers and on the inheritance of height of plants, number of nodes and leaves, flowering, and leaf size and shape. The results seem to justify the following conclusions:

1. The branching or suckering habit of tobacco is a distinct characteristic and its behavior in inheritance is similar to that of other inheritable characters, although it is subject to very considerable fluctuating variation due to environmental conditions and to certain physiological factors.

2. The F_1 generation of the cross between the variety with large and numerous suckers and the one with small and few suckers is intermediate between the two parents for number of suckers, although it is somewhat above the intermediate condition for weight of suckers, due no doubt to the stimulus of heterozygosis.

3. The reciprocal F_1 generations behave in an almost identical manner and the variation in this generation is on the whole no more than that of the most variable parent as shown by the standard deviation.

4. The F_2 generation possesses a range of variation as great or greater than that of the combined range of the two parents. The standard deviation from the mean as regards suckering habit is practically twice that of the average of the parents or of the F_1 . The large majority of the F_2 plants seem to approach more nearly the numerous-and-heavy-suckering type than they do the parent with few and small suckers, although this cannot be said with certainty as the means more nearly resemble the intermediate condition found in the F_1 .

5. Segregation in regard to suckering habit is definitely shown in the F_2 and succeeding generations. Strains have been isolated which sucker even less profusely than the Little Dutch parent, while others have been almost as prolific in this respect as the Cuban type. The large majority of the strains, however, range between the two extremes. The standard deviations show that certain of these strains are no more variable than the parents and are probably breeding true for suckering habit. Other strains, however, carried as far as F_6 , have failed to show any signs of the homozygous condition. The production of few and small suckers is seemingly the recessive condition.

6. The inheritance of suckering habit is purely quantitative and not separable into satisfactory classes or ratios. The results are comparable with those obtained by EAST, HAYES, and EMERSON for various quantitative characters in tobacco, maize, and other plants. It is believed the data should be interpreted in a similar way on the multiple factor hypothesis.

7. A considerable number of random observations and some systematically recorded data tend to show that no particular correlation exists between number, size, or shape of leaves, and the number or size of suckers in the second generation of a cross between a large-suckering and a small-suckering type. The production of a non-suckering type of tobacco combined with the commercial practice of "topping" is probably impossible for purely physiological reasons. It is believed, however, that by crossing and selection, the production of relatively-few-and-small-suckering strains of a certain type of leaf may be obtained which may be of some commercial importance; although it is probable that, on account of the number of factors involved, the combination of this characteristic with the proper leaf shape and quality will be extremely difficult to obtain.

8. Combination and segregation of characters has also been shown in this cross for such characters as height of plant, number of nodes and leaves, size and shape of leaves, and time of flowering.

9. The occurrence of two abnormalities entirely foreign to the parents, one a morphological monstrosity and the other a "physiological weakness," are recorded merely as illustrating the occurrence of "new" characters in a variety cross under controlled conditions.

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STUDIES ON SELF-STERILITY. III. THE RELATION BETWEEN SELF-FERTILE AND SELF-STERILE PLANTS

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INTRODUCTION

In the first paper of this series (EAST and PARK 1917), where the behavior of self-sterile plants was described in some detail, it was pointed out that the difference between self-fertile and self-sterile plants might prove to be a wholly different problem. This statement has been overlooked by several reviewers who criticized the interpretation of the behavior of self-sterile plants proposed because it failed to take into account the phenomenon of self-fertility. The prediction was not made at random, however. Even at that time various data had been gathered indicating a simple one-factor difference between self-fertile and self-sterile plants in keeping with COMPTON'S previous work (1912) on *Reseda odorata*. It can now be stated unequivocally that the position then taken is correct. In the material investigated self-fertile plants differ from self-sterile plants by a single essential Mendelian factor. Self-fertility is dominant. Adopting a presence-and-absence mode of expression, a plant is self-fertile because of the presence of a determiner for self-fertility; when this determiner is absent, the individual is self-sterile.

COMPTON'S WORK ON RESEDA

The only investigation in which crosses between self-fertile and self-sterile plants have been studied is that of COMPTON (1912, 1913) on the mignonette, *Reseda odorata*. Having had his attention directed to the species by the observations of Darwin, a number of experiments were made with the following results:

(1) Self-sterile plants intercrossed produced only self-sterile offspring. (2) Certain self-fertile plants when self-fertilized threw approximately 3 self-fertile to 1 self-sterile offspring. (3) These same plants when crossed with self-sterile individuals, produced self-fertiles and self-steriles in the ratio one to one. (4) Other self-fertile plants yielded none but self-fertile offspring from selfed seed.

These facts are satisfactorily interpreted by assuming a single factor difference with complete dominance. The recessives produced only recessives. The dominants in part produced only dominants and in part produced both types in the usual ratio of 3 to 1. He was dealing, therefore, in part with homozygous and in part with heterozygous plants, and the behavior of the heterozygous individuals was checked by the back cross with the recessive.

CORROBORATION OF COMPTON'S RESULTS BY EXPERIMENTS ON NICOTIANA

These experiments of COMPTON have been corroborated by crossing two of the self-sterile species used in our previous work, *Nicotiana Forgetiana* and *Nicotiana alata*, with a third species *Nicotiana Langsdorffii*, which is consistently self-fertile.

Nicotiana Forgetiana and *Nicotiana Langsdorffii* were crossed reciprocally. In each case the plants were very vigorous, exceeding both parents somewhat in height. They grew quickly, matured rapidly, and produced a profusion of fertile flowers. The flowers were somewhat intermediate in size but resembled the larger-flowered parent, *Nicotiana Forgetiana*, in form. No difference could be discerned in the reciprocals either in the first or second hybrid generation in appearance or behavior. The two experiments may therefore be considered as one.

About 400 plants were grown and selfed by hand with the usual precautions against cross-pollination. In each case, from 6 to 20 blossoms were operated on. *Every plant was self-fertile.* Seed set in abundance, filling the capsules. Not every flower pollinated produced seed, of course, but the percentage was practically the same as that obtained in check experiments on pure *Nicotiana Langsdorffii*, 85 percent. The work was completed as early in the season as possible in order not to be disturbed by the pseudo self-fertility which is sometimes present in self-sterile plants at the close of the flowering season.

From selfed seed of the cross *N. Forgetiana* \times *N. Langsdorffii*, 89 plants were grown and tested for self-fertility by guarded hand-pollina-

tions such as were made in the first hybrid generation. Of them 70 proved to be self-fertile and 19 self-sterile.

From selfed seed of the reciprocal cross, 92 plants were tested. Of this lot 74 showed self-fertility and 18 self-sterility. There was a sum total, therefore, of 144 self-fertile and 37 self-sterile plants in F_2 , a ratio 3.8 to 1.

If the hypothesis of a one-factor difference is correct the deficiency of recessives is somewhat greater than is to be expected in a population of this size. Nevertheless this failure to measure up to expectation need not disturb us. About one-fourth of the bags used in protecting the flowers were torn by wind, and the plants had to be tested a second time. This unfortunate occurrence prolonged the experiment until well into September when the plants were past their prime. It is not unexpected therefore that some truly self-sterile plants should have been listed as self-fertile because of "end-season" pseudo-fertility. In fact a slight fertility was shown by about 30 percent of the plants classed as self-sterile; i.e., they produced partially filled capsules in about 15 percent of the pollinations.

These plants were tested further by taking them into the greenhouse and bringing them into a second season of flowering. Pollinations were then made at the beginning of the season, and the plants proved to be fully self-sterile.

If this be not sufficient evidence to prove the case, there is the behavior of the third hybrid generation to be relied upon. *All progeny of the recessive (self-sterile) segregates of F_2 were again self-sterile.* About 200 were tested.

The cross between *N. Langsdorffii* and *N. alata* yield results similar to those just described. The plants of the first hybrid generation were all self-fertile; those of the second hybrid generation were partly self-fertile and partly self-sterile. About 200 F_2 plants were tested, of which 38 were self-sterile. Again there was a deficiency of recessives. The progeny of the self-steriles were all self-sterile, but no investigation of the amount of pseudo self-fertility was made. The matter of particular interest in this cross was the cross-fertility of F_2 plants having flowers of very different corolla lengths. Flowers were obtained as short as 2.0 cm and as long as 6.0 cm, yet reciprocal crosses were very easy to make.

It will be remembered that KÖLREUTER was unable to fertilize *Mirabilis longiflora* with pollen from *Mirabilis Jalapa* although the reverse cross could be carried out without difficulty. In interpreting these facts

it has been customary to assume that *M. Jalapa* pollen tubes are short and thus unable to reach the micropyles of the ovaries of *M. longiflora*. From work on pollen-tube growth (EAST and PARK 1918) and observations on the F_2 individuals of the cross between *N. Langsdorffii* and *N. alata*, we believe this assumption to be incorrect. Pollen tubes of all species observed by us have continued to grow as long as the flowers remained unwithered even in many generic crosses. The real cause of the occasional lack of success when a long-flowered plant is pollinated with pollen from a short-flowered plant, therefore, is in the "death" of the flower *before* the pollen tube has had time to reach the micropyle.

Though we may conclude that lack of a particular factor *F* results in self-sterility, there are some other factors to be considered in the behavior of crosses between self-fertile and self-sterile plants. When the self-sterile segregates of the cross between *N. Forgetiana* and *N. Langsdorffii* were examined carefully throughout the second flowering season, the type of self-sterility present did not seem to be the same in all cases. A majority of the plants exhibited a much greater amount of pseudo self-fertility than had ever been found in *N. Forgetiana*. In that species only an occasional plant produced a few selfed seeds and then only at the extreme end of the flowering season. Among the F_2 individuals of the cross, however, pseudo-fertility set in about the middle of the season and from then on it was very easy to get capsules which on casual examination would be said to be full of seed. As a matter of record only about 30 percent of such pollinations were successful and the capsules on the average had only about 70 percent of the normal complement of seed. Nevertheless, some 60 to 75 percent of the F_2 segregates classified as self-sterile showed at least 100 times the pseudo-fertility of the parent species, *N. Forgetiana*. The remaining plants were comparable to the latter in self-sterility.

It was also noticeable that the progeny of the most self-sterile of the F_2 plants were similar to them, while the progeny of the others were in part like their mother plants and in part like *N. Forgetiana*.

The simplest explanation of this state of affairs is that there is really a two-factor difference as regards self-sterility and self-fertility between *N. Forgetiana* and *N. Langsdorffii*. *N. Langsdorffii* is homozygous for a factor *F*; when this factor is absent the plants are self-sterile. It is also homozygous for a dilution factor *D*. The constitution of *N. Forgetiana* is *dd ff*. The F_1 individuals, having the constitution *Ff Dd*, are all self-fertile. In the F_2 generation a ratio of $9 FD : 3 Fd : 3 fD : 1 fd$ is

obtained. There are 3 self-fertile to 1 self-sterile because of the distribution of the allelomorphic pair F and f . But of the self-steriles, those having the constitution fD show a great deal more pseudo self-fertility than those having the constitution fd . Only the fd plants are wholly comparable to *N. Forgetiana*.

In describing the behavior of self-sterile plants this statement was made (EAST and PARK 1917):

"The waning of the reproductive period affects *N. alata* and *N. glutinosa* more markedly than it does *N. Forgetiana* or *N. angustifolia*. This indicates multiple allelomorphism in a fundamental factor the presence [or absence] of which is necessary for the development of self-sterility. This factor should not be confused with any of those assumed in the interpretation of the behavior of self-sterile plants among themselves."

The peculiarities of the cross between *N. Forgetiana* and *N. Langsdorffii* show that subsidiary factors affecting the manifestation of self-sterility, given homozygosity in ff , are as likely to be the interpretation of the differences shown in these four species as is multiple allelomorphism.

SUMMARY

Data are reported showing that in *Nicotiana* self-sterility is due to the presence of the allelomorph of a dominant fertility factor, F . When a population is homozygous for this factor, ff , it is self-sterile.

The factors which control the peculiar and systematic behavior of self-sterile plants when intercrossed among themselves are wholly independent of this factor and the latter does not need to be considered in an interpretation of their expression.

The manifestation of self-sterility as evinced by the degree to which pseudo-fertility shows, is due to a subsidiary inherited factor (or factors), but without the presence of the principal factor ff there is no evidence that it functions.

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STUDIES ON SELF-STERILITY. IV. SELECTIVE FERTILIZATION

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INTRODUCTION

Selective fertilization has been evoked many times as a means of accounting for peculiar or unusual breeding results. CASTLE'S (1903) original theory of sex-determination, and CUÉNOT'S (1908) interpretation of the non-appearance of homozygous yellow mice, are examples. Fortunately, it has always been possible to explain matters without retaining the hypothesis; in many cases, in fact, direct proof has been available that selective fertilization does not occur. Nevertheless, selective fertilization as a contingency has remained a sort of nightmare to investigators in genetics. Such antipathy is not unnatural, but one must have in mind the changes which have taken place in the subject during the last decade, to understand clearly the reason.

MENDEL'S discoveries, the Laws of Segregation and of Recombination, made heredity enticingly simple. All extensions, additions and exceptions have tended toward complexity. In this genetics has but repeated the history of chemistry and physics, yet it is to be expected, perhaps, that any suggested change in genetic conceptions savoring of increased complexity should find favor slowly. And selective fertilization is a tenet which would increase the difficulties of the subject a hundred-fold.

The outgrowth of Mendelism has been a theory of inheritance founded on the conception of specific character determiners, genes, located in the chromatin. In the sense that the central problem of heredity is clearly

one of chromosome, or at least of chromatin, distribution, the modern generalization has a simple grandeur not found in early Mendelism; but this simplicity is quite delusive, as a short consideration shows.

The conception of the gene is unquestionably the foundation of genetics. Students of heredity have submitted good evidence that *characters* are the product of many relatively *stable* genes which have a real basis in the germplasm, and that each of these genes may be the cause of various effects in different parts of the organism. They have shown that while the effects of a particular gene may not be wholly the same under different environmental conditions, nevertheless neither changes in the factors of environment nor association in particular combinations in the germplasm serves to change their individuality or constitution with a *significant* frequency.

Heredity, then, is the distribution of genes, and the genes have been located definitely in the chromosomes. Fortunately, chromosome distribution has been standardized in a remarkable manner in the majority of plants and animals; hence, the greater part of the phenomena found in breeding experiments may be described by a comparatively few simple mathematical formulae. It is to this orderly chromosome distribution that one must impute the utility of the Mendelian nomenclature, for to it in large measure is due the regularity with which certain ratios recur. There are irregularities in chromosome distribution, it is true. They have even furnished some of the critical tests of the modern theory of heredity taken as a whole. But because they curtail the practical value of the theory through limiting the possibilities of prediction, it is well that they are rare.

The standard chromosome mechanism for distribution of genes is that in which homologous chromosomes mate at synapsis, and homologous genes, *one* from either parent, pass by chance to either pole of the mitotic figure, in the formation of the mature gametes. The chromosomes may separate without having exchanged genes, presumably; or, genes may be exchanged. Just how this interchange occurs is not wholly clear. MORGAN has assumed that the genes have a linear arrangement, and that there must be transverse breaks in the chromosomes. CASTLE (1918) believes the arrangement is not linear, and that breaks may occur in many ways. It is possible that neither assumption is correct. The writer has felt for some time that possibly the genes are arranged spatially in a manner somewhat analogous to that assumed by chemists for organic molecules, though perhaps it might be better to say in a manner an-

alogous to certain crystals, for there certainly is no evidence that the genes are radicles belonging to single molecules. But the point is that with a spatial arrangement similar to that assumed for the radicles of molecules, with the homologous chromosomes mirror images of each other, with homologous genes interchanging by a definite mechanism, a more delicate system of action is possible than with mere chromosome breaks.

However this may be, the hinge on which the usefulness of this whole scheme turns is that *the genes pass to either daughter cell by chance*, and that the gametes thus formed *mate by chance*.

Even when such inheritance obtains, selective elimination of both gametes and zygotes is somewhat common, and causes rather chaotic conditions wherever it occurs. For example, the difficulties which characterize all endeavor to analyze inheritance in the *Oenotheras* are probably due in large measure to this cause. The additional difficulties which would arise should it be found that there is *selection of genes* in gamete formation, and *selection of gametes* at fertilization are so great as to be hardly imaginable.

DISCUSSION OF THE PROBLEM

Particularly suitable material with which to test the second possibility is found in those plants which are self-sterile. Since the direct cause of self-sterility is the slowness of growth of *self* pollen tubes as compared with *cross* pollen tubes, it would seem as if selective fertilization would have a better opportunity to manifest itself under such circumstances than under those which obtain in self-fertile plants and in animals.

Experiments with the self-sterile species *Nicotiana Forgetiana*, *N. alata* and *N. angustifolia* have shown that in self-pollinations and in incompatible cross-pollinations the pollen grains germinate as well as in compatible cross-pollinations. No differences are to be found between the two types either as to the percentage of grains germinating, the length of time required for germination, or the size of the tubes after germination, provided pollen tubes of the same length are measured. Pollen tubes produced after self-pollination or after incompatible cross-pollination grow so steadily that length plotted against time is a straight line; but pollen tubes produced after a compatible cross grow at such a constantly increasing rate that the growth curve resembles that of an autocatalytic reaction. As the flowering season is about to come to an end, more rapid pollen-tube growth occurs after a self-pollination or an in-

compatible cross-pollination, though there is little evidence of the accelerated growth characteristic of compatible combinations. The pollen tubes grow more rapidly, but the curve by its constant velocity still resembles the curve of a "normal" self-pollination.

These facts are the basis of our problem, and naturally they suggest the possibility of selective fertilization. Part of the work reported in the first of these studies (EAST and PARK 1917) was done upon a cross between *N. Forgetiana* and *N. alata*. The segregating generations naturally contained numerous individuals heterozygous for a large number of hereditary factors. There were differences in height of plant, size of leaf, color of flower, and size of flower, differences which could hardly be interpreted as the result of less than twenty or thirty determiners unless a great many of the variations shown in different organs were due to the activity of a single gene. Similar hereditary differences were marked even in the so-called pure species. This being true, it is important to know whether pollen tubes whose nuclei carry certain determiners grow faster than those which carry other determiners.

POLLEN-TUBE FREQUENCY DISTRIBUTIONS

One method which throws some light on the probability of selective fertilization is that of studying the frequency distribution of the pollen tubes after pollination. When applications of pollen are made, and the pistils prepared, sectioned and stained at varying periods of time after pollination, similar results are obtained no matter what the type of combination has been. In table 1, for example, a few frequency distributions of pollen-tubes after self-pollinating self-sterile plants during the height of the flowering season, are given. In general they are minus skew, and show that the greater number of pollen tubes are grouped at points from 3.5 mm to 7.5 mm from the end of the stigma at the expiration of from 5 to 7 days after pollination. A number of tubes have pushed out ahead of the majority, and a great many pollen grains—from 5 to 25 percent—have not germinated at all.

Distribution of pollen tubes in sections of pistils from self-sterile plants which had been pollinated at the end of the flowering season, show practically the same thing (table 2). The pollen tubes have reached distances comparable to those shown in table 1 in a shorter period of time, but otherwise no marked difference can be seen.

These two tables are presented merely for comparison with tables 3 and 4.

TABLE 1

Frequency distribution of pollen tubes after self-pollinating self-sterile plants during the height of the flowering season.

Source of data	Distance from the stigma in millimeters													
	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5
I section after 5 days.....	2	20	25	20	14	6	5	2						
I section after 6 days.....		1	43	30	30	20	13	7	7	5	2	2	1	
I section after 6 days.....	2	2	50	41	40	16	17	6	5	2	3	1	1	
I section after 7 days.....					4	20	24	18	9	7	2	2	1	
I section after 7 days.....			1	6	20	25	21	16	16	8	6	2	1	1

TABLE 2

Frequency distribution of pollen tubes after self-pollinating self-sterile plants at the end of the flowering season

Source of data	Distance from the stigma in millimeters													
	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5
I section after 3 days.....	3	3	20	30	16	6	2	1	1	1				
I section after 3 days.....		6	30	21	20	18	6	2	2	1				
I section after 4 days.....		1	6	20	28	16	8	4	5	2	1			
I section after 4 days.....			2	6	18	25	26	24	20	16	8	2	1	
I section after 4 days.....						2	18	20	18	8	6	4	3	1

In table 3 some distributions of pollen tubes from the F_2 generation of a cross between *N. Forgetiana* and *N. alata* are given. The cross is compatible, and since the individuals are unquestionably heterozygous in a large number of factors, they should show a marked tendency to vary if there is selective fertilization. The frequency distributions shown in table 4, on the other hand, where *sib* matings for three generations ought

TABLE 3

Frequency distribution of pollen tubes after cross-pollinating compatible plants of F_2 generation *N. Forgetiana* \times *N. alata*.

Source of data	Distance from the stigma in millimeters											
	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5	19.5	20.5	21.5
1 section after 2 days.....	3	16	26	28	24	21	16	12	8	2	1	1
1 section after 2 days.....	6	20	21	16	8	3	1					
1 section after 2 days.....			1	16	20	18	16	8	3	3	1	
1 section after 3 days.....				1	16	15	14	8	4	2	2	2
1 section after 3 days.....			1	6	40	28	20	16	3	1	1	

TABLE 4

Frequency distribution of pollen tubes after cross-pollinating compatible plants of F_2 generation (sib matings) *N. Forgetiana* \times *N. alata*

Source of data	Distance from the stigma in millimeters											
	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5	19.5	20.5	21.5
1 section after 2 days.....		6	18	24	20	18	7	2	4	2		
1 section after 2 days.....			2	36	30	20	18	7	6	5		1
1 section after 3 days.....			1	16	26	21	17	5	5	2	1	
1 section after 3 days.....				2	24	21	18	3	8	1	2	
1 section after 3 days.....					1	16	26	21	20	14	10	4

to have brought about a considerable degree of homozygosis, should be less variable. As a matter of fact, however, there seems to be no significant difference in the two cases.

There is no evidence that variability in gametic constitution is the cause of variability in rate of pollen-tube growth. In fact, there is no positive proof that there is a measurable variability in pollen-tube growth.

In both of these types of pollination and in all similar cases examined, percentages of ungerminated pollen grains comparable to those determined for incompatible matings were found. The actual percentages have little meaning, for ungerminated pollen grains are loosely held by the stigmas and the correct number of grains which do not germinate is not likely to be obtained. But the fact that a considerable percentage of grains which contain protoplasm and in every respect seem to be normal, remain as long as 6 days without germinating, leads one to believe that difference in the rate of germination is largely responsible for the varied length of the pollen tubes measured. The pollen grains may differ among themselves in the thickness of their walls or the composition of the protoplasm outside the nuclei, thus accounting in some measure for rapidity of germination, without it being necessary to assume gametic differentiation as a cause. Furthermore the entire series of results on the behavior of self-sterile plants reported in the first paper of this series (EAST and PARK 1917), makes it unlikely that differences in gametic composition show themselves in any way *before fertilization*. The factorial composition of the mother plant controls the behavior of self-sterile plants, and all the pollen grains of a single plant may be taken to have the same factorial composition as far as any functions to be performed *before* fertilization are concerned.

It is not to be supposed that the variability in length of pollen tube shown in tables 3 and 4 really represents the difference of time at fertilization. In compatible matings the pollen tubes grow faster and faster so that the variability shown in a frequency distribution of pollen tubes determined at 1 day or 2 days after pollination may be quite different at a later date. It has not been found possible to obtain satisfactory measurements of pollen tubes as they approach the micropyles, but it may be assumed that at this time the rate of growth is so fast that practically all of the ovules are fertilized within a few hours. Selective fertilization is hardly probable therefore for this additional reason.

INFLUENCE OF THE GROWTH OF COMPATIBLE POLLEN TUBES UPON INCOMPATIBLE POLLEN TUBES

In interpreting the results of our experiments on pollen-tube growth (EAST and PARK 1918), it was assumed that after a compatible cross substances are secreted in the pistil which accelerate the elongation of the tube, and that the immediate cause of this secretion is a catalyser which the pollen-tube nucleus is able to produce because the hereditary consti-

tution of the plant producing it is different from that of the plant on which it is placed. Superficial consideration might lead one to suppose that if this were true, incompatible pollen tubes would be accelerated by the growth of compatible pollen tubes if a mixture of the two kinds of pollen were placed on the stigma. Second thought, however, shows that this is probably not the case. Plant enzymes are colloids having large molecules, hence they do not pass freely through cell membranes. Their actions are largely local; where they do not seem to be local, the direct cause of the reaction is more likely to be a crystalloid produced by action of the colloid.

The writer has been able to devise no experiment to measure absolutely such possible stimulation, but two experiments have shown that when mixtures of compatible and incompatible pollen are applied to a single stigma, only the compatible pollen produces seed.

In the first experiment a number of pistils were pollinated with a definite number of compatible pollen grains. The work was done under a binocular, and the count is thought to be accurate within an experimental error of ± 2 grains. The pistils were then carefully covered with incompatible pollen. Eight capsules matured with the results shown in table 5.

TABLE 5
*The effect of compatible pollen on the growth of
incompatible pollen tubes.*

Pistil No.	Number of compatible pollen grains	Number of seeds produced
1	51	46
2	48	42
3	50	41
4	62	49
5	32	23
6	67	58
7	61	54
8	46	40

The indications from this experiment are that no incompatible pollen tubes contributed to the production of the seeds obtained; but of course it is impossible to maintain that these tubes were not accelerated in their growth to some degree.

In the second experiment, a more critical test of the matter was made. Three pistils of a white-flowered self-sterile plant coming from a line of

plants homozygous for this color were selfed. Five or six hours after these plants were covered with pollen from a self-sterile family bearing red flowers. Capsules full of seed were obtained. If these seeds were produced by the compatible pollen only, the resulting progeny should be red-flowered for red is dominant; if incompatible pollen has functioned, white-flowered plants should be obtained. Three hundred plants have been grown with *not a single* white-flowered individual.

SUMMARY AND DISCUSSION

The experiments described in this paper were designed to test the possibility of selective fertilization occurring in self-sterile *Nicotianas*, it being assumed that from the nature of the material the phenomenon might here be possible. (1) Comparisons were made between the pollen-tube frequency distributions of highly heterozygous and of comparatively homozygous plants. (2) The influence of compatible matings on incompatible matings was investigated. In neither case was there any indication of selective fertilization.

Though it is impossible to prove a negative, there is so much circumstantial evidence against selection both in the formation of gametes and zygotes, the probability that it ever occurs is very remote. In the first place gametes are formed in many animals and plants, particularly in species crosses, which can never function. If the mechanism of gamete formation were such as to make it necessary to assume a selection of genes, a low frequency of non-functional gametes would be expected. Similarly zygotes are produced in the numbers to be expected by chance mating of gametes, even though these zygotes have no possibility of passing through a complete life cycle. There are two cases in mice, eight in *Drosophila*, and four in plants where the evidence of lethal factors is too complete to be disregarded. In reality there are probably hundreds of such instances in plants and animals which have been investigated during recent years that ought to be interpreted in the same manner.

Again, pollen grains show no tendency to behave as if the genes which they carry function before fertilization. It will be recalled that BATESON (1909) found pollen shape and color in the sweet pea to be inherited as a maternal character. The writer (EAST 1916) has corroborated this discovery for color of *Nicotiana* pollen. It may be claimed, however, that these facts are just what is to be expected because of the morphogenesis of the outer characters of the pollen grain. This is true; but

the criticism does not apply to the phenomena found in the behavior of self-sterile plants in cross matings where cross-sterility of groups of plants exists *presumably because of genes possessed by the mother plants*. In fact the only activity shown by a male gametophyte which seems to be due to the factors it is carrying over into the next generation, is a lack of any activity. In BELLING's (1914) work on the velvet bean, he found 50 percent of the F_1 pollen was abortive in a certain cross. It appears then that in this instance the presence or absence of a gene of the generation which would ordinarily function after fertilization, has caused the pollen grain to abort. This lack of ability to function does not necessarily mean the actual activity of the genes of this generation however; the machine has simply remained uncompleted, so to speak. For this reason, there seems to be no wisdom in even suspecting selective fertilization; unless mixtures of pollen (or spermatozoa even) from different individuals should be used. If pollen grains from a single plant are alike as far as their activities before fertilization are concerned, there is no basis for selection.

May we not extend this conception to animals for the present and accept as a fundamental genetic hypothesis the tenet of chance segregation in the germ cells and chance mating of these germ cells?

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STUDIES ON SELF-STERILITY V. A FAMILY OF SELF-STERILE PLANTS WHOLLY CROSS-STERILE *INTER SE*

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In the first paper of this series (EAST and PARK 1917),¹ the behavior of a number of families of self-sterile plants under various schemes of mating was described. In one cross between the two species *Nicotiana Forgetiana* and *Nicotiana alata*, fifty-three plants of the F₁ generation could be separated into not less than four groups in which each member of every group was cross-sterile with every other member of that group, although showing cross-fertility with every member of every other group. The interpretation given of these and other similar facts was in brief as follows: (1) a self-sterile species exhibits this peculiarity because homozygous for a basic self-sterility factor; (2) a series of partially coupled factors affects the behavior of sterile plants among themselves; (3) these secondary factors act as if sporophytic in nature, so that all gametes produced by a single individual are identical in this regard with the plant on which they originated; (4) the nature of the action of these secondary factors is such that two plants are not fertile together unless they differ by at least one of these factors.

Though the self-sterility and the cross-sterility existent in these plants is fully expressed at the beginning and height of the flowering season, toward the close of the flowering season, particularly in plants exhibiting the effect of adverse environmental conditions, occasionally some fertility is shown. It is possible therefore to obtain seed from truly self-sterile plants and from combinations that are fundamentally incompatible. By taking advantage of this pseudo-fertility it should be possible to obtain families of plants wholly cross-sterile *inter se*. Such a family, apparently, is family E described on pages 565 to 567 of the paper we are discussing. Unfortunately very little work had been done on this family when that paper was written. Since a reserve supply of the seed

¹ EAST, E. M., and PARK, J. B. Studies on self-sterility I. The behavior of self-sterile plants. *Genetics* 2: 505-609, 1917.

from which the family came had been preserved, however, it has been possible to make a more extended study of the case.

In table 1 are recorded the infertile crosses made upon 54 plants of

TABLE I

Record of infertile crosses made on 54 plants of family E(2). Number of pollinations is shown by subscripts.

Ped. No.	Sterile with Ped. No. ♂	Sterile with Ped. No. ♀
1	3 ₃ , 5 ₃ , 10 ₃ , 50 ₃ , 75	44 ₄ , 57 ₃ , 59 ₃ , 61 ₃ , 72 ₄ , 73 ₃ , 74 ₃
3	5 ₅ , 8 ₃ , 14 ₃ , 23 ₂ , 50 ₃	1 ₂ , 5 ₃ , 69 ₃
5	3 ₃ , 9 ₄ , 10 ₅ , 14 ₃ , 16 ₃ , 23 ₃ , 50 ₃ , 74 ₂	1 ₃ , 3 ₅ , 54 ₃ , 55 ₃ , 56 ₇ , 57 ₃ , 59 ₄ , 60 ₃ , 61 ₁ , 74 ₇
8	9 ₃ , 10 ₃ , 14 ₃ , 16 ₃ , 17 ₃ , 50 ₃	3 ₃
9	10 ₃ , 14 ₃ , 16 ₃ , 17 ₃ , 23 ₃ , 50 ₃	5 ₄ , 8 ₃ , 61 ₃
10	14 ₃ , 17 ₃ , 44 ₃ , 50 ₃	1 ₃ , 5 ₅ , 8 ₃ , 9 ₃ , 45 ₃ , 58 ₃ , 61 ₃ , 67 ₃ , 68 ₃
14	16 ₃ , 17 ₃ , 19 ₃ , 26 ₃ , 43 ₃ , 50 ₁₁ , 75 ₃	3 ₁₁ , 5 ₃ , 8 ₃ , 9 ₃ , 10 ₃ , 50 ₂ , 73 ₄
16	17 ₂ , 19 ₃ , 41 ₃ , 42 ₃ , 44 ₃ , 50 ₃	5 ₃ , 8 ₃ , 9 ₃ , 14 ₃
17	22 ₃ , 23 ₃ , 43 ₃ , 44 ₃ , 50 ₃ , 55 ₃	8 ₃ , 9 ₃ , 10 ₃ , 14 ₃ , 16 ₂
19	23 ₄ , 26 ₄ , 29 ₃ , 50 ₃ , 61 ₃ , 62 ₃ , 69 ₄ , 75 ₃	14 ₃ , 16 ₃
22	26 ₃ , 27 ₃ , 31 ₄ , 50 ₃ , 61 ₃ , 62 ₃ , 69 ₃	17 ₃
23	26 ₄ , 41 ₃ , 42 ₃ , 44 ₂ , 50 ₃ , 56 ₃	3 ₂ , 5 ₃ , 9 ₃ , 17 ₃ , 19 ₄ , 53 ₃ , 54 ₄ , 56 ₃ , 63 ₃ , 64 ₃ , 66 ₃ , 72 ₃ , 73 ₃
26	31 ₄ , 35 ₄ , 38 ₃ , 50 ₃ , 61 ₃ , 62 ₃	14 ₃ , 19 ₄ , 22 ₃ , 23 ₄ , 42 ₃ , 44 ₃ , 49 ₃ , 55 ₃
27	29 ₃ , 35 ₃ , 50 ₃ , 61 ₄ , 62 ₃ , 75 ₃	22 ₃
29	31 ₃ , 33 ₃ , 38 ₃ , 50 ₃ , 61 ₄ , 62 ₃ , 68 ₃	19 ₃ , 27 ₃ , 75 ₄
31	33 ₃ , 35 ₄ , 38 ₃ , 50 ₃ , 61 ₄ , 62 ₃ , 73 ₃	22 ₄ , 26 ₃ , 29 ₃ , 42 ₃ , 43 ₃ , 75 ₃
33	35 ₃ , 38 ₃ , 39 ₇ , 40 ₇ , 41 ₇ , 50 ₃	29 ₃ , 31 ₃ , 38 ₄ , 75 ₃
35	38 ₃ , 39 ₃ , 40 ₄ , 42 ₃ , 50 ₃ , 61 ₃ , 69 ₃ , 75 ₃	26 ₄ , 27 ₃ , 31 ₄ , 33 ₃ , 68 ₃ , 70 ₃ , 71 ₃ , 73 ₃
38	33 ₄ , 39 ₃ , 40 ₄ , 42 ₃ , 44 ₃ , 50 ₄ , 61 ₃ , 62 ₄ , 73 ₄	26 ₃ , 29 ₃ , 31 ₃ , 33 ₃ , 33 ₃ , 35 ₃ , 52 ₃ , 75 ₃
39	40 ₄ , 42 ₃ , 43 ₃ , 50 ₇ , 60 ₃ , 61 ₃ , 62 ₃ , 75 ₄	33 ₇ , 35 ₃ , 38 ₃ , 44 ₃ , 49 ₃ , 50 ₃ , 52 ₃ , 63 ₃ , 64 ₃
40	42 ₃ , 44 ₄ , 45 ₃ , 50 ₃ , 52 ₃ , 61 ₄ , 62 ₃ , 63 ₄	33 ₇ , 35 ₄ , 38 ₄ , 39 ₄

TABLE I (continued)
Record of infertile crosses made on 54 plants of family E (2). Number of pollinations is shown by subscripts.

Ped. No	Sterile with Ped. No. ♂	Sterile with Ped No. ♀
41	44 ₃ , 46 ₃ , 52 ₃ , 53 ₃ , 54 ₃ , 74 ₃	16 ₃ , 23 ₃ , 33 ₇ , 43 ₃
42	26 ₃ , 31 ₃ , 44 ₃ , 50 ₃ , 75 ₃	16 ₃ , 23 ₃ , 35 ₃ , 38 ₃ , 39 ₃ , 40 ₃ , 43 ₄
43	31 ₃ , 41 ₃ , 44 ₃ , 50 ₃ , 59 ₃	14 ₃ , 17 ₃ , 39 ₃ , 61 ₄
44	1 ₄ , 26 ₃ , 39 ₃ , 46 ₃ , 49 ₃ , 50 ₃ , 52 ₃ , 53 ₃ , 54 ₄	10 ₃ , 16 ₃ , 17 ₃ , 23 ₂ , 38 ₃ , 40 ₄ , 41 ₃ , 42 ₃ , 43 ₃ , 45 ₃ , 50 ₁₃ , 52 ₃
45	10 ₃ , 44 ₃ , 46 ₃ , 50 ₃ , 61 ₄	40 ₃ , 49 ₃
46		41 ₃ , 44 ₃ , 45 ₃
49	26 ₃ , 39 ₃ , 45 ₃ , 50 ₄ , 51 ₃ , 62 ₃	44 ₃
50	14 ₂ , 39 ₃ , 44 ₁₃ , 52 ₃ , 53 ₄ , 54 ₃ , 56 ₃	1 ₃ , 3 ₃ , 5 ₃ , 8 ₃ , 9 ₃ , 10 ₃ , 14 ₃ , 16 ₃ , 17 ₃ , 19 ₃ , 22 ₃ , 23 ₃ , 26 ₃ , 27 ₃ , 29 ₃ , 31 ₃ , 33 ₃ , 35 ₃ , 38 ₄ , 39 ₇ , 40 ₃ , 42 ₃ , 43 ₃ , 44 ₃ , 45 ₃ , 49 ₄ , 52 ₃ , 53 ₃ , 54 ₃ , 55 ₃ , 56 ₃ , 57 ₄ , 58 ₃ , 59 ₃ , 60 ₃ , 61 ₃ , 63 ₃ , 64 ₃ , 65 ₃ , 66 ₃ , 67 ₃ , 68 ₃ , 69 ₃ , 70 ₃ , 71 ₃ , 72 ₃ , 74 ₃ , 75 ₃
51		49 ₃
52	38 ₃ , 39 ₃ , 44 ₃ , 50 ₃ , 53 ₄ , 54 ₃ , 55 ₄ , 56 ₃ , 57 ₃ , 58 ₃ , 59 ₃ , 68 ₄	40 ₄ , 41 ₃ , 44 ₃ , 50 ₃
53	23 ₃ , 50 ₃ , 54 ₄ , 55 ₃ , 56 ₃ , 57 ₃ , 58 ₄ , 62 ₃ , 64 ₃	41 ₃ , 44 ₃ , 50 ₄ , 52 ₄
54	5 ₃ , 23 ₄ , 50 ₃ , 55 ₃ , 56 ₄ , 57 ₃ , 58 ₃ , 64 ₃	41 ₃ , 44 ₄ , 50 ₃ , 52 ₃ , 53 ₄
55	5 ₃ , 26 ₃ , 50 ₃ , 56 ₃ , 57 ₃ , 58 ₃ , 59 ₃ , 62 ₄	17 ₃ , 52 ₄ , 53 ₃ , 54 ₃
56	5 ₇ , 23 ₃ , 50 ₃ , 57 ₃ , 58 ₃ , 59 ₃ , 60 ₃ , 61 ₃ , 63 ₄ , 64 ₂	23 ₃ , 50 ₃ , 52 ₃ , 53 ₃ , 54 ₄ , 55 ₃
57	1 ₃ , 5 ₃ , 50 ₄ , 58 ₃ , 59 ₄ , 60 ₃ , 61 ₃ , 69 ₄	52 ₃ , 53 ₃ , 54 ₃ , 55 ₃ , 56 ₃
58	10 ₃ , 50 ₃ , 59 ₃ , 60 ₃ , 61 ₃ , 65 ₄ , 73 ₃ , 74 ₄	52 ₃ , 53 ₄ , 54 ₃ , 55 ₄ , 56 ₃ , 57 ₃
59	1 ₃ , 5 ₄ , 50 ₃ , 60 ₃ , 61 ₃ , 71 ₃ , 73 ₃ , 75 ₃	43 ₃ , 52 ₃ , 55 ₃ , 56 ₃ , 57 ₄ , 58 ₃
60	5 ₃ , 50 ₃ , 61 ₃ , 69 ₃ , 71 ₃ , 75 ₄	39 ₃ , 56 ₃ , 57 ₃ , 58 ₃ , 59 ₃
61	1 ₃ , 5 ₃ , 9 ₃ , 10 ₃ , 43 ₄ , 50 ₃ , 69 ₃ , 71 ₄	19 ₃ , 22 ₃ , 26 ₃ , 27 ₄ , 29 ₄ , 31 ₄ , 35 ₃ , 38 ₃ , 39 ₃ , 40 ₄ , 45 ₄ , 56 ₃ , 57 ₃ , 58 ₃ , 59 ₃ , 60 ₃ , 63 ₃ , 64 ₃ , 65 ₃ , 66 ₃ , 74 ₃ , 75 ₄
62		19 ₃ , 22 ₃ , 26 ₃ , 27 ₃ , 29 ₃ , 31 ₃ , 38 ₄ , 39 ₃ , 40 ₃ , 49 ₃ , 53 ₃ , 55 ₄ , 63 ₃ , 64 ₃ , 69 ₃ , 71 ₄ , 72 ₃ , 73 ₃ , 75 ₄

TABLE I (continued)

Record of infertile crosses made on 54 plants of family E (2). Number of pollinations is shown by subscripts.

Ped. No	Sterile with Ped. No. ♂	Sterile with Ped No. ♀
63	23 ₃ , 39 ₃ , 50 ₃ , 61 ₃ , 62 ₃ , 64 ₃ , 65 ₃ , 74 ₃ , 75 ₃	40 ₄ , 56 ₄ , 73 ₃ , 74 ₄
64	23 ₃ , 39 ₃ , 50 ₃ , 61 ₃ , 62 ₃ , 67 ₃ , 68 ₃ , 73 ₄ , 75 ₃	53 ₃ , 54 ₃ , 56 ₂ , 63 ₃ , 65 ₃ , 66 ₃ , 69 ₃ , 70 ₃ , 71 ₄ , 73 ₃
65	1 ₃ , 50 ₃ , 61 ₃ , 64 ₃ , 68 ₃ , 69 ₃ , 70 ₂	58 ₄ , 63 ₃
66	23 ₃ , 50 ₃ , 61 ₃ , 64 ₃ , 71 ₃ , 73 ₃ , 74 ₄	67 ₂ , 68 ₃
67	10 ₃ , 50 ₃ , 67 ₂ , 69 ₃ , 70 ₃ , 71 ₃	64 ₃
68	10 ₃ , 35 ₃ , 50 ₃ , 66 ₃ , 69 ₃ , 70 ₃	29 ₃ , 52 ₄ , 64 ₃ , 65 ₃ , 69 ₃
69	3 ₃ , 50 ₃ , 62 ₃ , 64 ₃ , 68 ₃ , 73 ₃ , 74 ₃ , 75 ₃	19 ₄ , 22 ₃ , 35 ₃ , 57 ₄ , 60 ₃ , 61 ₃ , 65 ₃ , 67 ₃ , 68 ₃ , 70 ₄ , 71 ₃ , 75 ₃
70	35 ₃ , 50 ₃ , 64 ₃ , 69 ₄ , 71 ₃ , 72 ₃ , 73 ₄ , 74 ₃	65 ₂ , 67 ₃ , 68 ₃
71	35 ₃ , 50 ₃ , 62 ₄ , 64 ₄ , 69 ₆ , 72 ₃ , 73 ₃ , 74 ₃	59 ₃ , 60 ₃ , 61 ₄ , 66 ₃ , 67 ₃ , 70 ₃ , 72 ₄
72	1 ₄ , 23 ₃ , 50 ₃ , 62 ₃ , 71 ₄ , 73 ₃ , 74 ₃	70 ₃ , 71 ₃ , 73 ₄
73	1 ₃ , 14 ₄ , 23 ₃ , 35 ₃ , 62 ₃ , 63 ₃ , 64 ₃ , 72 ₄	31 ₃ , 38 ₄ , 58 ₃ , 59 ₃ , 64 ₄ , 66 ₃ , 69 ₃ , 70 ₄ , 71 ₃ , 72 ₃ , 74 ₄
74	1 ₃ , 57 ₃ , 50 ₃ , 61 ₃ , 63 ₄ , 73 ₄ , 75 ₃	52 ₂ , 41 ₃ , 58 ₄ , 63 ₃ , 66 ₄ , 69 ₃ , 70 ₃ , 71 ₃ , 72 ₃
75	29 ₄ , 31 ₃ , 33 ₃ , 38 ₃ , 50 ₃ , 61 ₄ , 62 ₄ , 69 ₃	1 ₁ , 14 ₃ , 19 ₃ , 27 ₃ , 35 ₃ , 39 ₄ , 42 ₃ , 59 ₃ , 60 ₄ , 63 ₃ , 64 ₃ , 69 ₃ , 74 ₃

this family, the subscripts showing the number of attempts made for each combination. The table was constructed by assuming that if a combination had been made one way, the reciprocal had also been made, as explained in our former study (EAST and PARK 1917). Thus it can be seen that while only a fraction of the possible combinations were made, nevertheless the plants were linked together in an unbroken chain. In other words, if it be true that when A is sterile with B and with C, B is sterile with C, then each of these 54 plants is sterile with the other.

It is not true however that no seed at all was obtained in the numerous attempts to combine plants of this family. Table 2 shows that 13 combinations produced capsules. From the number of sterile pollinations made with the same plants and from the fact that nearly all of the fertility appeared at the end of the flowering season, it would seem that

TABLE 2

*Record of fertile crosses made on 54 plants of family E (2),-
presumably pseudo-fertility. First number is female.*

Combination	Number of fertile pollinations	Number of sterile pollinations	Number of sterile reciprocal pollinations
3 × 5	1	5	3
5 × 10	1	5	—
5 × 74	1	2	See 74 × 5
16 × 50	1	8	—
17 × 22	1	3	—
23 × 44	1	2	—
33 × 38	2	6	4
39 × 50	4	7	3
44 × 50	1	6	See 50 × 44
44 × 52	1	5	3
50 × 44	1	13	See 44 × 50
56 × 23	1	8	3
74 × 5	1	7	See 5 × 74

these apparent exceptions are all illustrations of fluctuating pseudo-fertility. There is the whole of our experience with this type of fertility back of such an assertion, but there is also some specific evidence on the case in point.

The number of seeds produced by these plants when crossed with compatible pollen is in general from 300 to 600 per capsule (table 3), while the number of seeds in the presumably pseudo-fertile combinations is usually much less. At the same time 4 of the latter combinations produced what seemed to be full capsules. Combination 16 × 50 produced a full capsule at the seventh attempt, although eight out of nine attempts were failures, and combination 50 × 44 produced a full capsule at the twelfth attempt although thirteen out of fourteen attempts were failures. On the other hand plant 23 gave a full capsule with pollen of plant 44 on the first attempt, plant 33 gave two capsules out of eight attempts with pollen of plant 38, and plant 39 gave four capsules out of eleven attempts with pollen of plant 50. Now combination 33 × 38 was about 50 per cent fertile, and combination 39 × 50 became progressively more fertile as shown by the number of seeds produced. These three plants, 23, 39, and 50 were crossed with a large number of other plants, nevertheless, and showed cross-sterility. Further, at the beginning of another flowering season crosses 23 × 44 and 39 × 50 were impossible. At the same time it is not without the bounds of probability that combination 39 × 50

TABLE 3

Comparison of the number of seeds in capsules of the presumably pseudo-fertile combinations in family E(2) with the number of seeds in the capsules of the same plants when pollinated with pollen from the plant of the F₂ generation of the cross between N. Forgetiana and N. Langsdorffii.

Combination	Number of seeds in capsules	Number of seeds in capsules when pollinated with (314 × 328)
3 × 5	28	
5 × 10	115	365, 382, 421
5 × 74	127	365, 382, 421
16 × 50	418	
17 × 22	27	370
23 × 44	436	
33 × 38	124, 131	
39 × 50	151, 255, 289, 352	
44 × 50	128	
44 × 52	191	
50 × 44	427	
56 × 23	82	
74 × 5	185	

was for some unknown reason more easy to make than other combinations in this family. We have no theory to offer at present as to why this may be true. It may stand as an open question. The general conclusion from all the evidence is that family E (2) may be considered to consist of plants wholly cross-sterile *inter se*.

The question then arises: Is the origin of family E (2) compatible with our previous conclusions as to the behavior of self-sterile plants when crossed *inter se*. First, it must be emphasized that the cross-sterility found has nothing to do with true sterility. A random sample of 25 plants was used in a test with the pollen a single plant coming from the F₂ generation of a cross between *Nicotiana Forgetiana* and *Nicotiana Langsdorffii*. Out of 64 pollinations there were only 2 failures (table 4). Again, out of 51 attempts to use the pollen of these plants in crosses thought to be compatible, there was only 1 failure. The sterility found, therefore, is wholly of the nature termed "self-sterility," or "incompatibility," and must be interpreted as such.

The origin of a family consisting of one class of plants cross-sterile with each other was to have been predicted on the basis of the interpre-

TABLE 4

Record of pollinations made on a random sample of 25 plants of family E(2) with pollen from a single self-sterile plant of the F₂ generation of a cross between Nicotiana Forgetiana and Nicotiana Langsdorffii (814 × 328).

Plant No.	Successful pollinations	Unsuccessful pollinations	Number of seeds in each mature capsule
5	3	0	365, 382, 421
6	3	0	360, 330, 515
9	4	1	390, 500, 400, 370
10	1	0	631
17	1	0	370
22	3	0	358, 405, 230
25	1	0	377
26	2	0	630, 425
31	3	0	481, 404, 470
36	5	0	453, 350, 405, 372, 340
38	2	0	432, 192
40	3	0	271, 195, 317
46	1	1	185
51	4	0	225, 252, 382, 384
53	1	0	157
54	2	0	420, 177
57	4	0	295, 650, 250, 462
58	3	0	700, 618, 902
59	1	0	327
60	1	0	330
61	5	0	635, 588, 580, 468, 678
64	3	0	338, 230, 376
70	2	0	230, 176
71	2	0	480, 240
73	2	0	291, 358
Total	62	2	

tations we have used, by taking advantage of the phenomenon of pseudo self-fertility. Continued self-fertilization is possible by persistent efforts at self-pollination carried to the very end of the flowering season. And continued self-fertilization should bring about homozygosis in the secondary factors affecting the behavior of self-sterile plants among themselves. When such a point is reached, the resulting population should not only be self-sterile but should belong to a single class all members of which are cross-sterile with each other.

Family E (2) was not the result of continued self-pollination and pseudo self-fertility, however. It was produced as follows: The fe-

male parent was No. 58, a plant of *N. alata*, the result of three generations of selfing a self-sterile strain *at the end of the season*. The behavior of No. 58 and of its sister plants when crossed with each other leads one to believe they were all members of one intra-sterile class, but the evidence is hardly sufficient to establish the point. The male parent was a member of the F_1 population (plant 44, *loc. cit.*, p. 559) produced by crossing a self-sterile plant of *N. Forgetiana* with a sister plant of the mother of the *N. alata* plant just described (No. 58).

Now it is obvious that the female parent of this family may have come from a fraternity homozygous for the secondary factors effective on compatibility *inter se*. They may have been, for example, plants with the composition *AABB*. It is possible also that the male parent, though originating from a cross, might have had the formula *AABB*, since its parents might have been *AABB* and *AaBB*. But a whole population having a single formula could not have arisen through a cross except through an illegitimate mating (pseudo-fertility). If then the two parents of the population had the same constitution and produced seed through pseudo-fertility, then family D (*loc. cit.*, p. 563), coming from the same male crossed on a sister of plant 58, ought to be a duplicate of family E (2). This however does not appear to be the case. Family D consisted of at least two intra-sterile classes, unless a good deal of unrecognized pseudo-sterility was present. On the other hand both of the parents, in the few tests made on family E (2), were sterile with their progeny,—a result to be expected on the theory of homozygosis. It seems, then, that the unsettled question, a question which must await further investigation, is, why family D and family E (2) are not similar in composition and behavior.

SELECTION OF PSEUDO-STARCHY ENDOSPERM IN MAIZE

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INTRODUCTION

Endosperm differences in maize were among the first utilized in testing the generality of MENDEL'S doctrine of the independent segregation of unmodified determiners. They served as material for the early investigations of DE VRIES, CORRENS, LOCK and WEBBER. Among the characters employed, the starchy and sweet types of seed formed a useful contrasting pair, as the difference was marked, segregation was distinct and in practically every case the inheritance clearly followed the rule of the monohybrid. More recently conditions have been described and pictured which seem to indicate that this simple state of affairs in the endosperm of maize does not always exist. Semi-starchy endosperms more or less intermediate in appearance between true starchy and true sweet types have been noted and where the inheritance of such conditions has been followed no clear-cut segregation has been observed.

Some of this anomalous material appeared after a cross between a starchy and a sweet variety of maize. A possible inference from this has been that a contrasting pair of factors, which usually segregate distinctly, may sometimes affect each other, while in hybrid union, in such a way that segregation is not complete and a re-arrangement of hereditary substances is thereby brought about resulting in new and unstable effects. This state of affairs has been spoken of as contamination of genes. Cases of this kind have been supposed to occur in other material and the whole matter has been a subject for some dispute which has considerable im-

portance, since incomplete segregation or contamination of factors, if it does exist, would seriously limit the usefulness of Mendelian formulas. The investigation to be reported here was undertaken in the endeavor to throw some light on this problem by determining the relation of this new endosperm condition to the ordinary starchy type of seed and to find out the response of this semi-starchy character to selection.

EAST and HAYES (1911) in a publication dealing with inheritance of many characters in maize, reported a large number of crosses involving the starchy and sweet type of endosperm, and found in practically every case that segregation was precise and that no unusual deviations from a monohybrid ratio were obtained. The recessive continued true to type when recovered and tested, except in one instance. This occurred in the progeny of extracted sweet seeds from a cross of dent (EAST and HAYES No. 8, Illinois High Protein strain) by sweet (No. 54, Black Mexican sweet). All but three ears came true to the type of typical recessive sweet seeds. These three unusual ears are described as semi-starchy. One of them is shown in the publication referred to (plate III b, p. 40), and is compared with a pure extracted sweet ear from a sister plant, and with a pure extracted starchy ear. As illustrated the seeds of this semi-starchy ear appear intermediate in the amount of opaqueness and shrinking of the endosperm as compared with the other two ears which may be taken to represent the parental types which went into the cross, as far as the endosperm texture is concerned.

A typical sweet ear, such as the one pictured in the illustration of EAST and HAYES, has the seeds deeply wrinkled and their surfaces strongly contorted with rough, angular projections caused by a pronounced shrinking of the contents of the seeds on drying. In addition to the large depressions, fine wavy markings on the surface of the seeds may also be observed. Sweet seeds are more or less translucent, allowing the outlines of the embryo to be seen, and have a hard, brittle texture of a glassy nature accentuated by lines of fracture which frequently occur. The appearance of sweet seeds is modified by the size and shape of the seeds. Varieties of latent dent type differ considerably from those of latent flint type. Lying next to the embryo a thin layer of opaque white material can be seen, when the seed is broken, which resembles the floury starch of dent varieties. When examined under the microscope, however, the starch grains are unequal in size, irregular in outline and are accompanied by a large amount of amorphous substances making the separation of the grains difficult.

Starchy varieties of maize include several different types with respect to the texture and shape of the seeds. They range from the hard translucent seeds of the pop and flint varieties (*Z. mays everta* and *indurata*) to the soft, opaque seeds of the floury type (*amylacea*). Both soft and hard starch occur together in dent seeds (*indentata*) where the typical indented tip is caused by unequal contractions of the two kinds of starch. The surface of the seeds of all starchy varieties is smooth without the wrinkling and folding of the outer layers and in this respect the two types of maize are generally quite distinct. All classes of starchy maize differ from sweet varieties in the fact that the starch grains, particularly from the white opaque portions of the seed, are large, plump, nearly round in outline and show characteristic markings. It is this ability to complete the development of the starch grains that forms the principal hereditary difference between starchiness and sweetness. This is shown noticeably in chemical composition. Sweet seeds, because they have a lower starch content, have proportionately higher percentages of other ingredients—sugar, protein, fat, ash and fiber. Their high sugar content gives them their name, and is the reason why they are preferred as a vegetable. Their ability to produce sugar has undoubtedly been selected for until the total production in amount of this substance has been increased to a greater extent than in field varieties.

Somewhat recently a type of endosperm coming from China, differing from both sweet and starchy, has been described. This is called waxy and has a peculiar tough consistency distinguishing it from other kinds of maize. Sweet and waxy are complementary in their action in inheritance so that when the two are crossed starchy endosperm is produced. They can be considered as endosperm deficiencies—one lacks one thing; the other lacks something else—both of which are necessary for normal starch production.

The semi-starchy seeds obtained by EAST and HAYES (1911) differed from the pure sweet segregates in having solid areas of opaque white varying in amount and in location in various parts and in different seeds on an ear. But this substance was most noticeable at the center of the seeds, surrounding the embryo, and at the tips of the seeds, at the point of attachment of the stigmas. The seeds were distinctly wrinkled but the surface was not so rough as in the case of the typical sweet seeds. The diameter of the starch grains was compared to the conditions of the two normal segregates (EAST and HAYES's table 10, p. 45) and in this respect the semi-starchy type was found to be intermediate. As men-



FIGURE 1.—Result of selecting for the most starchy-appearing individual during ten generations of self-fertilization in pseudo-starchy material originating from an extracted sweet seed out of a cross of starchy and sweet.



FIGURE 2.—Result of selecting for the most sweet-appearing individual during nine generations of self-fertilization from the same source as in figure 1.

tioned before there was some variation on the ear in the amount of opaque substances in the semi-starchy seeds. The most starchy- and the least starchy-appearing seeds from one of these ears were separated and planted. Some of the resulting ears from these two selected lots of seed are shown in the same publication (plate IV). No great differences are to be observed between these two progenies. Apparently selection in one generation had very little visible effect, although the starchy selection shows slightly more of the opaque white areas than the sweet selection. From these two lines the most starchy ear and the most sweet ear were selected and grown each year thereafter, the plants in practically every case being self-fertilized.

The original cross of dent by sweet (8×54) was made in 1907. The semi-starchy ear appeared in the F_2 plant generation in 1909 grown from a sweet seed out of an F_2 segregating starchy-sweet seed population. The first selection was made from the one ear that year and the selection continued to the present time (1918) so that the material on hand represents the result of selecting for two extremes, starchy and sweet, during 10 generations and, in all, 11 generations of self-fertilization after the original cross. This material is not well suited for a selection experiment as hand-pollinated ears only can be used. From 10 to 15 such ears were produced every year in each of the two lines. All of the 1915 seed of the sweet line failed to germinate in 1916 so that seed of the previous year had to be planted and one generation was lost in this way.

The selection and growing of the material was done at the start under the direction of Dr. E. M. EAST and later of Professor H. K. HAYES up to the year 1915 when the experiment came into the hands of the writer who wishes to acknowledge his indebtedness for the material and the outline of the methods of carrying on the experiment. The writer is particularly under obligation to Dr. EAST for his examination of the material for the past several years, for his help with the interpretation of the results and criticism of this report.

After 10 years of selection in the starchy line and 9 years in the sweet line the two resulting types are shown in figures 1 and 2. These ears were grown in 1917 but are like the ones grown the past year so may be used to represent the conditions at the close of the period of selection. It will be seen in these reproductions that the ears of both lines are as different in appearance as any sweet and flint varieties. The ears of the sweet selection vary somewhat in the amount of opaque substance,

but on the whole are as clear and wrinkled as most varieties of the latent flint type and more so than many. On the other hand the seeds of the most starchy-appearing ear of the other selection are perfectly smooth, plump and wholly opaque without patches of wrinkling or translucence. Other ears of this starchy line show the effect of some shrinking but for the most part are quite smooth and opaque. The depressions where they occur are in the form of dimples rather than angular distortions on the surface although there are areas on a few seeds which closely resemble the wrinkled condition of sweet seeds.

The three ears of both lots represent fairly well the range of variation. These two selections have come true to these types during the four years that they have been under my observation. Just when they first appeared as they now are and as constant, the records do not show. A few ears of both lines grown in 1913 after 5 years of selection are available and show as great differences as at the present time. Whether or not all the ears were as different as these, that is, whether the variation was any greater at that time, is not known. The differences between individual ears are so slight that no statistical way of presenting the fluctuations within the lines or the distinction between the two has been attempted. Since there was little response in the first generation apparently selection brought about the maximum differences in four generations. After the first five generations changes have been slight until during the last four or five years no visible alterations have taken place.

It is apparent, therefore, that selection operating with this semi-starchy character which originated in a rather questionable manner, has had a profound effect on the end results. Types visibly as different as the two parents which were crossed have been recovered from an intermediate condition suggesting incomplete segregation of the determining factors. Without other evidence to judge from one might be justified in saying that here is a case of a blending of heredity factors. The blend has been variable and from it by selection parental types have been gradually re-established and stabilized. Nowhere is there evidence of definite segregation. Fluctuation is the rule until constancy is obtained only after several years of selection. Even the usual interpretation of quantitatively variable characters as due to many determiners meets with a hindrance here as in such cases selection, after crossing, is followed by an immediate response and the effect is greatest right at the first.

When these two types, which are visibly so different, are crossed with any true starchy variety the beginning of an understanding of the true

situation is made. The selected sweet strain fertilized with starchy-carrying pollen shows perfect dominance of starchiness and segregation in the next generation, as expected, in the 3 to 1 starchy-sweet ratio. The selected starchy strain pollinated from the same source shows no dominance of starchiness because it is already starchy *in appearance*, but in the next generation it segregates 3 to 1, *starchy* and *sweet*, just like the first cross. This is enough to make us suspect the nature of the starchy-appearing strain. Upon examination, the starch grains of this pretending amylaceous selection are still far from being the same as those of true starchy seeds. In this respect dominance is shown in the cross just mentioned. From its behavior in heredity, and because of the difference of the starch grains this condition of the endosperm of maize that we are dealing with is called pseudo-starchy. This character is considered to be distinct genetically from true starchiness and no particular significance is attached to the fact that it appeared after a cross of garden and field types. In its inheritance it is independent of true starchiness. Moreover it occurs in other types of sweet maize which so far as known have not been crossed with the dominant endosperm. Thus selection is as far from reproducing true starchiness at the close of the experiment as it was at the start and segregation of the two principal allelomorphs in the original cross was exact and not accompanied by any unusual processes. This pseudo-starchy character will now be described more in detail as well as its behavior when crossed with true sweet and true starchy endosperms.

One of the principal points of difference between the three types of endosperm is to be found in the starch grains. In figures 3, 4, and 5 are shown the magnified particles obtained from starchy seeds, from seeds of the pseudo-starchy selection and from the sweet selection. The seeds were soaked in weak alcohol for about ten days, then cut in half and a smear obtained by pressing the cut surfaces against a glass slide. This material was stained with iodine, dried and mounted in balsam. The size and condition of the grains is comparative. The starchy seeds used were of the dent type, large, well developed with considerable areas of white, floury starch. Most of the starch grains in the illustration came from these areas as such grains separated the most easily when the smears were made. As can be seen these grains are large and nearly round in outline. Those from the wrinkled sweet seeds are small, indistinct, irregular in outline and most of them are many-sided, being pentagonal or hexagonal. They are accompanied by a large amount of amor-

phous substances which causes the grains to aggregate. Large particles are shown in the photograph which are probably not single grains but groups. The smear from the pseudo-starchy selection resembles more nearly that from the sweet seeds than from the starchy seeds, but the grains are more distinct and larger. They are quite angular, giving the impression that they have been tightly packed together.

When typical seeds of the pseudo-starchy selection are cut into they are found to be brittle and in this respect very much like typical sweet seeds. At the center, above the embryo, there is usually a cavity surrounded by a small area of opaque white substance resembling floury starch, but there is a marked difference in consistency. In true starchy seeds the floury portions can be easily scraped out and are much like chalk. In the pseudo-starchy seeds the white areas are tough and cannot be easily separated from the other parts of the seed. When examined under the microscope the differences are as shown in figures 3 and 4. Around the white area and making up the bulk of the endosperm is a hard layer having much the same texture as sugary endosperm but more opaque.

The principal visible difference between pseudo-starchy and sweet seeds is that the former on drying shrink in the center and contract towards an outer hull which retains its shape, whereas in sweet seeds all parts contract towards the center on drying leaving the surface contorted and irregular. In behavior the sweet and pseudo-starchy seeds are considerably alike. Both are more subject to decay while maturing than starchy seeds although they differ in this respect, the sweet seeds being more susceptible.

In chemical composition pseudo-starchiness and true starchiness are more nearly alike as shown by the results given in table I. These figures were obtained under the direction of Dr. E. M. BAILEY in the analytical laboratory of the CONNECTICUT EXPERIMENT STATION. The determinations are averages of closely agreeing duplicates and have been made according to the official methods of analysis. The results for the starchy and sweet types agree fairly well with other published data (PEARL and BARTLETT 1912) in everything but the soluble and insoluble carbohydrates. It is thought that these differences are largely due to the methods of analysis and that the three determinations given in the table are comparative. It should be remembered that the results represent only a gross chemical analysis. Great differences may exist and not be shown by similarity in percentage content of soluble and insoluble carbohy-

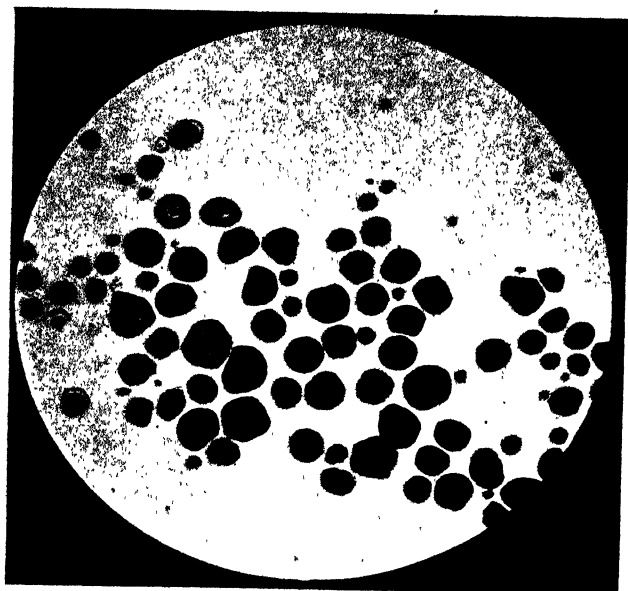


FIGURE 3.—Starch grains from true starchy seeds.

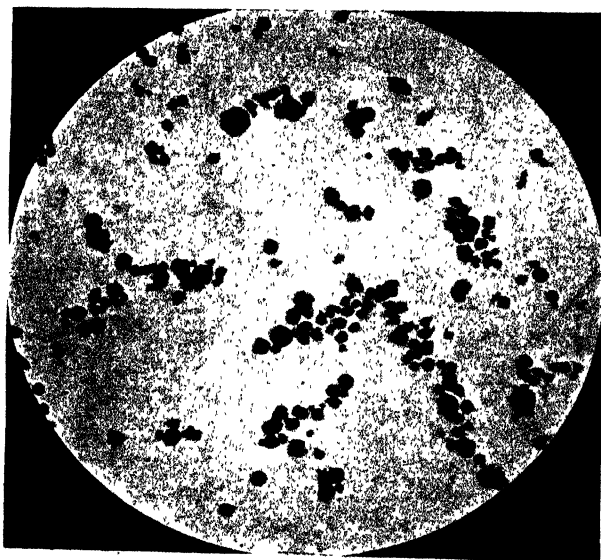


FIGURE 4.—Starch grains from pseudo-starchy seeds.

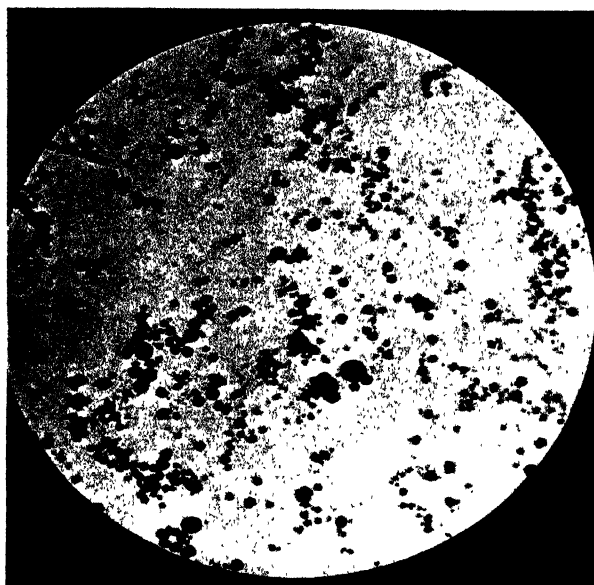


FIGURE 5.—Starch grains from sweet seeds.

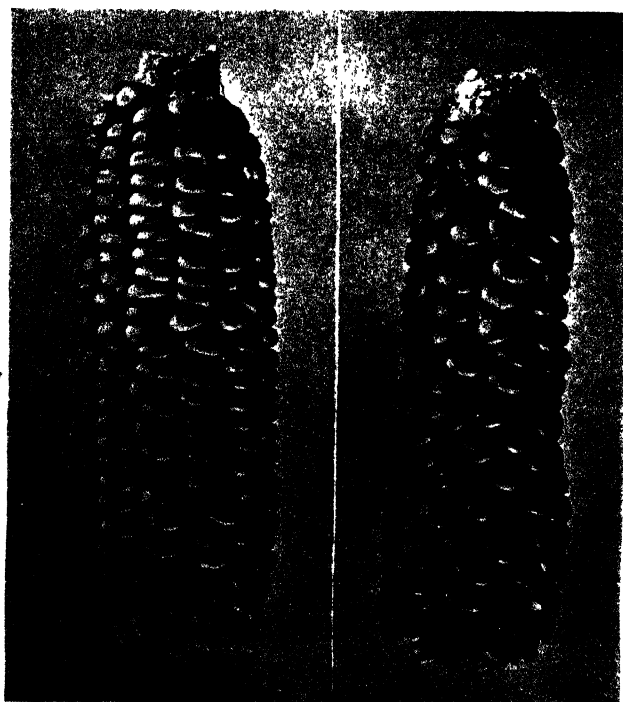


FIGURE 6.—Pseudo-starchy ears showing a few anomalous translucent seeds.

TABLE I

Chemical composition of pseudo-starchy seeds compared to true starchy and sweet seeds.

Ingredients	Sweet	Pseudo-starchy	Starchy
Moisture	10.60	11.09	11.41
Ash	2.07	1.65	1.46
Protein	14.51	12.69	11.60
Fiber	1.89	1.73	1.31
Fat (crude)	7.52	5.94	4.07
Total soluble carbohydrates as dextrose....	26.15	7.19	4.45
Insoluble carbohydrates, starch.....	29.00	51.50	58.73
Undetermined nitrogen-free extract by difference	8.26	8.21	6.97
	100.00	100.00	100.00

drates. As far as the figures are significant the pseudo-starchy maize is intermediate in moisture content, somewhat more like sweet maize in percent of fiber, crude fat and undetermined nitrogen-free extract. In ash, protein and particularly soluble and insoluble carbohydrates the pseudo-starchy type approaches very closely to the condition of true starchy maize.

Summing up these statements it can be said that in external appearance and in gross chemical analysis the pseudo-starchy type is much like starchy maize. In the nature of the starch grains and texture of the endosperm it is more like sweet maize. In its behavior in crosses it is wholly unlike the true starchy character and in this respect differs also from pure sweet endosperm as will be shown later.

This pseudo-starchy endosperm seems to be entirely unlike the waxy endosperm described by COLLINS and KEMPTON (1913). No crosses have been made between the two types as yet, but the brittle, glassy texture is quite unlike the tough consistency of waxy seeds. This pseudo-starchy is apparently a new kind of maize to be added to the long list of types based on endosperm constitution. STURTEVANT (1899) described semi-starchy ears for which he proposed the specific name of *Zea amyleasaccharata*. He had only three specimens upon which to base his description. They showed an intermediate condition probably similar to the original ear from which selection was started in this experiment. There is hardly a doubt but that STURTEVANT was dealing with the same character, although not so highly intensified, as in the selected material described here.

OCCURRENCE OF ANOMALOUS ENDOSPERM IN OTHER MATERIAL

This character in a partially developed condition occurs quite commonly; more or less of it can be found in nearly every variety of sweet corn. It is found sometimes rather highly developed and causes the seedsmen and sweet corn growers some concern. Just what effect this character has upon the quality of the product for table purposes is undecided. Chemical analysis indicates that it is undesirable. The percent of soluble carbohydrates (sugars) is reduced from 26.15 in the sweet selection to 7.19 in the pseudo-starchy material. But many sweet varieties, highly esteemed for their "quality" (which usually means high sugar content) show considerable amounts of this pseudo-starchiness, notably Golden Bantam and Black Mexican. The latter variety often has large quantities of this substance which is obscured by the colored aleurone.

The starchy-appearing character is most pronouncedly developed in the extremely early varieties of sweet corn. Early Dawn, Malakhov and other early ripening sorts have some seeds which are almost entirely opaque but still show some wrinkling. Apparently this character increases the resistance of the seeds to decay, helps the germination of the seeds and the hardiness of the seedling plants grown from them. There is a notable difference in this respect between sweet and starchy maize. There is also a difference between the sweet and pseudo-starchy seeds in this respect but not so great. As stated before, all the seed of the sweet selection failed to germinate one year although the pseudo-starchy seeds germinated well. In the production of early, hardy varieties of sweet corn this pseudo-starchy character undoubtedly has value. This earliness and hardiness, however, are obtained with some sacrifice of sugar content.

HALSTED (1909) has observed the same character and has practiced selection in open-pollinated cultures. The starchiness first appeared in a cross of two sweet varieties, Malakhov and Premo. The material resulting from this cross he called "Malamo." After selecting for starchy-appearing ears without hand pollination for several years, he obtained one ear which was practically solid flinty in appearance. From this ear he grew 66 ears of which 34 were flinty and 32 generally sweet. The sweet extremes were nearly like pure sweet. Flint extremes were found with a few slightly wrinkled kernels as were ears showing all gradations between the two extremes. His conclusions in regard to the matter are as follows:

"A study of the whole set of ears leads one to feel that in some in-

stances the flintiness or its absence seems to indicate a plant character, for in some ears the sweet grains are the rare exceptions and in others the flinty ones are scarce. Sometimes an ear will have all its grains in a semi-flinty condition. Again, one may be led to the opinion that the grain in its starch unum acts quite independently of its neighbors, for among a large number of smooth ones will be a strongly wrinkled one."

Although HALSTED was not working with controlled cultures there is a close agreement between his results and our own. The character is quantitatively highly variable and responds to selection. It is controlled by heredity determiners which seem to be partly plant factors and partly seed factors.

A point to be noted in regard to HALSTED's results is that this character arose in a cross of two sweet varieties, not with a starchy variety as was the case in the present experiment. Yet, if he had practiced selection with controlled pollinations he would undoubtedly have obtained as highly developed and as pure a starchy strain as we obtained, because many of his individual seeds and ears showed this character fully developed. Of course, all varieties of sweet corn are subject to more or less crossing with field varieties, and it is possible that HALSTED's material was so crossed previous to his taking up selection with it, but the impression is reinforced from his observations that there is no necessary relation between the pseudo-starchy character and true starchiness.

The last statement of HALSTED's referring to the occurrence of a few strongly wrinkled seeds among many seeds which are smooth, brings up another matter the discussion of which has been reserved to this point. In our starchy selection ears were obtained which were perfectly smooth and opaque with exception of one, two, or several translucent and slightly wrinkled seeds. The difference between the two kinds of seed was abrupt, there being no transitional seeds upon the ears. Figure 6 shows two such ears, one with five seeds and the other with one seed of this kind. Not all of the ears obtained each year showed these seeds but usually several did. During the last four years such ears have been avoided in selecting the individual for continuing the starchy line. In 1917 one ear was obtained which had several translucent seeds. These were planted and from them one selfed ear was secured. This is like its parent ear. All the seeds are smooth, plump and perfectly opaque with the exception of two translucent seeds one of which is slightly wrinkled. These two seeds are perfectly distinct from the others. No other ears which contained seeds of this kind were found in the starchy selection that year. Apparently this phenomenon is the same or similar

to what sometimes occurs in true starchy maize. The writer has obtained one self-pollinated ear of true flint type with three translucent wrinkled seeds resembling sweet seeds in a total of some three or four hundred seeds. Such a proportion could hardly be a chance deviation from a 3 : 1 segregation on the supposition that the plant which produced the ear was accidentally crossed by sweet-carrying pollen in the year previous. Neither can it be a segregation distorted by lethal factors, unless such factors act upon the pollen alone, as the ear is fully developed with no missing seeds.

Various hypotheses have been advanced to shed light on the occurrence of anomalous-endosperm seeds in maize. Whether they are due to somatic mutation, cytological aberrations or unknown causes still remains in doubt. The subject has been reviewed by EMERSON (1918). It is found that most of the anomalies of this kind occur in hybrid seeds. In this pseudo-starchy material and the flint ear just mentioned, we are dealing with a homozygous type or very nearly such. The translucent condition of the endosperm does not correspond with the embryo because such seeds reproduce the normal type. Yet the *tendency to produce the aberrancy* seems to be inherited in some fashion since the number of the seeds has become less in the later generations of selection until in the last year no ears with off-type seeds were obtained except in one case in which the parent seed was itself off-type. The material under consideration has not been sufficiently investigated in this connection to warrant any further treatment of the matter. The occurrence of these peculiar seeds will be considered as a problem in itself and as unrelated to the variability of the ears as a whole with respect to the degree of development of the pseudo-starchy character.

BEHAVIOR OF PSEUDO-STARCHINESS IN CROSSES

Since from six to ten generations of self-fertilization is ordinarily sufficient to bring about nearly complete homozygosis, confidence can be placed in the belief that the plants which were used in the crosses represent fairly pure types. The crosses were made in 1915 after the parents had been self-fertilized eight years. The plants were quite uniform and considerably reduced in size and vigor. This fact furnished a very reliable check on the accuracy of the results since whenever foreign pollen gains access to the silks by accident such out-crossed plants the next year show a great increase in size and vigor so that they cannot be possibly mistaken. Moreover the leaf characters and plant habit are so characteristic that unintentional pollinations can be detected with a high

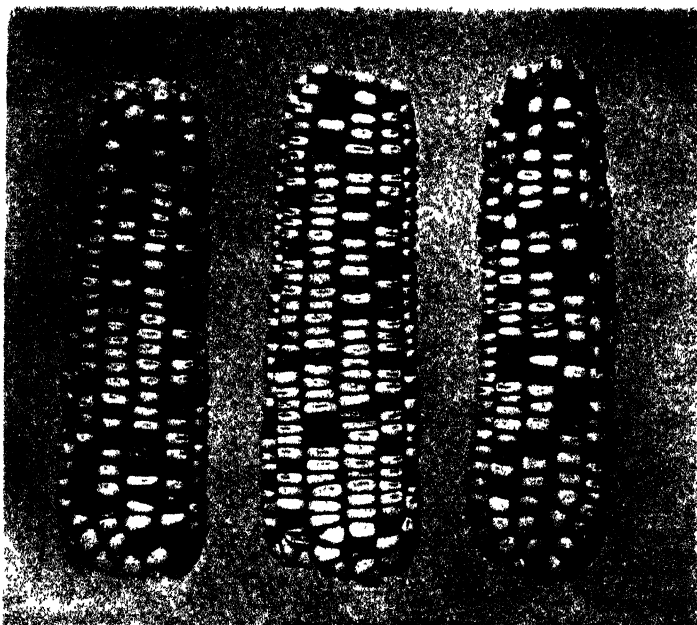


FIGURE 7.—F₂ seed populations from a cross of true starchy by sweet.

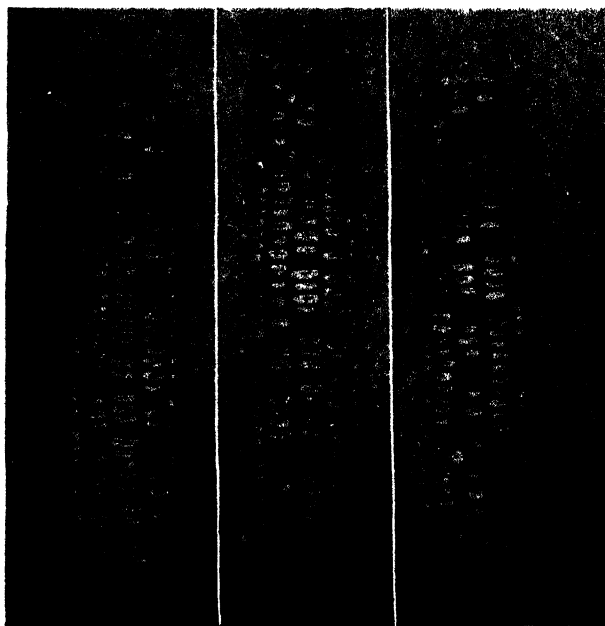


FIGURE 8.—F₂ seed populations from a cross of true starchy by pseudo-starchy.

degree of surety. Not many of such illegitimate plants were found in the course of this experiment but a few seem to be unavoidable.

When pseudo-starchy and true starchy plants are crossed the result in the second generation is, at first sight, exactly the same as when sweet and starchy are crossed as has been stated before. The numbers obtained from reciprocal crosses and back-crosses are given in table 2 and show

TABLE 2

The number and ratio of starchy and sweet seeds obtained in F_2 and in back-crosses from reciprocal crosses of pseudo-starchy and true starchy endosperm.

Second generation selfed	Starchy	Sweet	Total	Ratio per 4	Deviation	Probable error
Starchy \times pseudo-starchy, F_2	1832	550	2382	3.0764: .9236	0.0764	0.0239
Pseudo-starchy \times starchy, F_2	1918	761	2679	2.8638: 1.1362	0.1362	0.0226
Total	3750	1311	5061	2.9638: 1.0362	0.0362	0.0164
Back-crosses with sweet				Ratio per 2		
(Starchy \times pseudo-starchy) \times sweet	832	779	1611	1.0329: .9671	0.0329	0.0168
Sweet \times (starchy \times pseudo-starchy)	494	468	962	1.0270: .9730	0.0270	0.0218
(Pseudo-starchy \times starchy) \times sweet	1280	1401	2681	.9549: 1.0451	0.0451	0.0130
Sweet \times (pseudo-starchy \times starchy)	241	310	551	.8748: 1.1252	0.1252	0.0287
Total	2847	2958	5805	.9809: 1.0191	0.0191	0.0124

no marked deviations from the expected ratios. Segregation is definite and the ears look like those obtained from the other cross. Samples of these two lots of ears are shown in figures 7 and 8. Instead of the pseudo-starchy endosperm coming out in F_2 as it went into the cross it appears as sweet endosperm. When these wrinkled segregates are examined closely, however, they are seen to contain more of the opaque substances than similar seeds of the other cross. Apparently true starchiness and pseudo-starchiness are genetically independent of each other and the former has such a pronounced effect that it overwhelms, for the time being, whatever differences there may be between pseudo-starchy and sweet endosperms. It is important to note that the factors brought in by the starchy parent greatly alter the size and shape of the seeds and for this reason cause the new endosperm character to be very much reduced in expression and in fact almost obliterated.

When the two selected lines, sweet and pseudo-starchy, are crossed reciprocally, very little immediate effect can be seen. In this respect also pseudo-starchiness differs from true starchiness. When the sweet

selection is used as the female parent the seeds show faint traces of opaqueness, usually at the tips of the seeds, but the whole effect is far from an intermediate condition. The reciprocally crossed seeds show slightly more shrinking and approach the sweet condition about as far as in the reverse case. The self-pollinated ears grown on F_1 plants, either way the cross is made, are distinctly intermediate with a large range of variation in the seed populations on an individual ear as well as among the ears themselves. Three F_1 ears selected to show the range of variation between different ears and in the seeds on an ear, are reproduced in figure 9. It can be seen that one ear is almost entirely sweet, one is nearly starchy with some appearance of segregation and one is distinctly segregating but no sharp differences between individual seeds can be seen. All gradations from smooth opaque to wrinkled translucent seeds can be found on this ear. The dark-colored seeds on these ears have been attacked by mold. The differences between the individual seeds, it can be assumed, are due to genetic differences in endosperm factors coupled with some environmental variation. The differences between individual ears cannot be so easily considered to be due to genetic differences since they are produced on F_1 plants and the parents have been assumed to be homozygous. Of course, the fact that the parental lines were self-pollinated for a number of generations does not insure homozygosity. MULLER (1918) has demonstrated ways by which enforced heterozygosity may be maintained by various systems of lethal factors, particularly when two different lethal factors balance each other by being in homologous chromosomes. When this condition occurs together with factors which reduce or prevent crossing over, almost every conceivable kind of unusual results can be produced. While some such state of affairs may exist in this material no definite evidence of this has been found. Moreover maize is particularly favorable material in which to detect lethal factors, as the whole progeny of a plant is produced in an inflorescence where commonly all the seeds are placed in regular order. All lethals which destroy the female gametes or stop development immediately after the zygote is formed, show up at once in the missing seeds whose places are distributed throughout the ear. Self-pollinated ears showing regular vacancies are sometimes found and warrant further study but as far as known nothing of this kind occurs in this material under investigation. Lethal factors which destroy male gametes or which prevent the germination of seeds could not be easily detected. It seems more logical, however, to attribute the variations in F_1 ears to

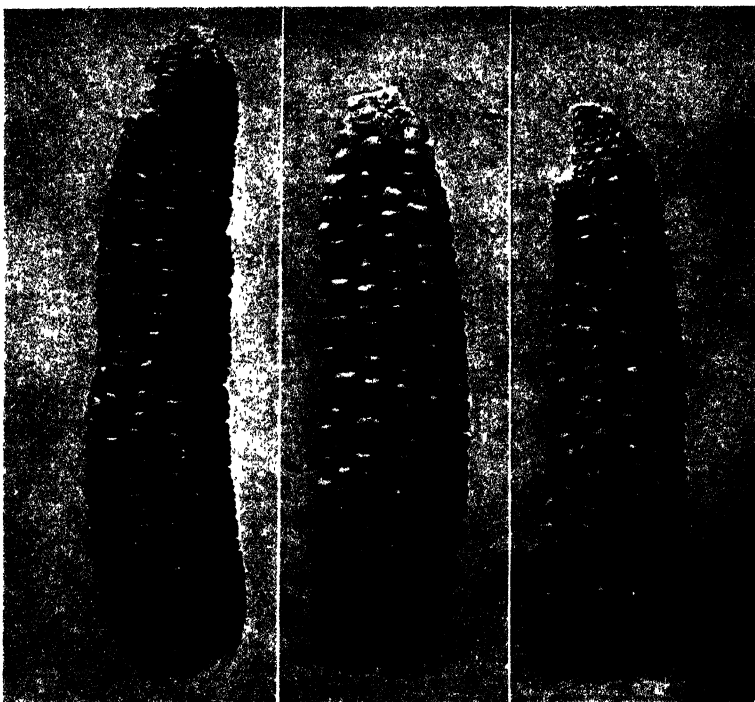


FIGURE 9.—F₂ seed populations from a cross of sweet by pseudo-starchy (dark seeds are discolored by mold).

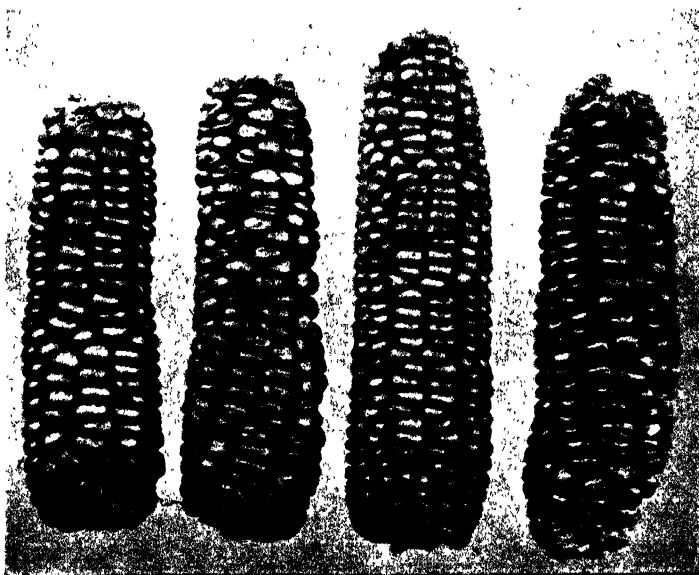


FIGURE 10.—Result of selecting the most starchy-appearing seeds from an F₂ seed population from a cross of sweet by pseudo-starchy.

external effects. Since there is some variation in the parental types, variability in F_1 plants is also permissible, but to the eye the amount of variation is somewhat greater. Differences in time of ripening affect this endosperm condition which is rather unstable. Individual ears are found with the tip seeds more wrinkled and translucent than those at the center of the ears. This non-genetic variability may be greater in the heterozygous than in the homozygous condition.

From F_1 ears like the one in the center of figure 9, which gave the greatest indication of segregation, seeds were selected which showed the most development of opaqueness and seeds which showed this least developed. Selection was made from two such ears. The self-pollinated ears grown from one of these individuals are shown in the accompanying illustrations: the progeny from the starchy selected seeds in figure 10 and from the sweet selected seeds in figure 11. The results from the other ear were similar to those two lots. Selection of the extremes in F_2 seed populations grown on F_1 plants, brings about small visible differences in the next generation. On the whole, however, the most translucent ear of the sweet selection differs considerably from the most opaque ear of the starchy selection. Apparently endosperm factors have something to do with the development of the character under investigation.

None of these ears grown on F_2 plants gave any more evidence of distinct segregation than did the F_1 ears. Some showed segregation more than others, however. This fact is not clearly brought out in the reproductions. The seeds from two segregating ears—one from the sweet, one from the starchy selection—were again separated into most starchy- and most sweet-appearing and grown the following year. Also from the two F_2 plant progenies which had been selected for sweet one ear from each was chosen for seed which most nearly approached the original sweet parent. Likewise from the two F_2 plant progenies selected for starchiness one ear from each was taken for planting which most nearly approached the original starchy parent. This procedure and the results obtained may be outlined as shown in diagram 1.

Since the gradations between individual seeds and between different ears are so small and indefinite there is no good statistical method of presenting the data. In the diagram the progenies resulting from the selections are lettered so that they can be referred to. The results obtained can be briefly stated.

The immediate reciprocal crosses are without any marked effect in

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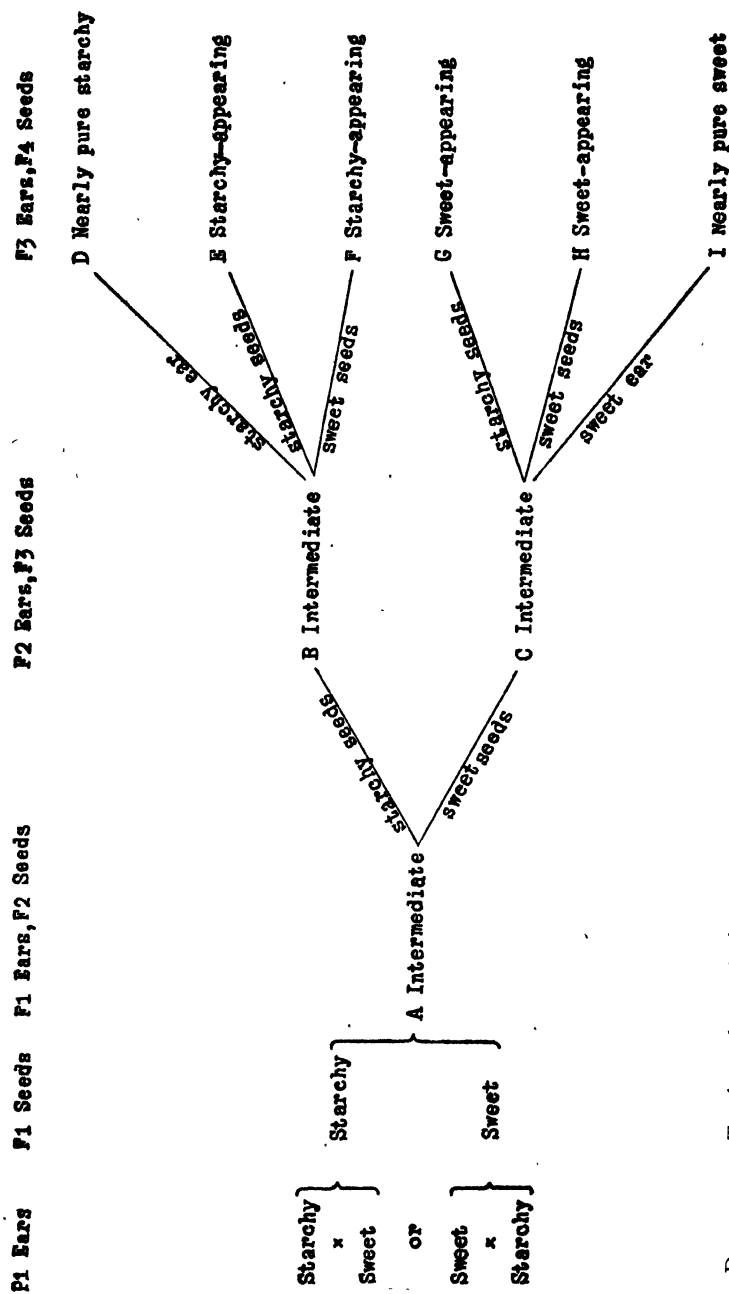


DIAGRAM 1.—To show the method and progress of selection after crossing pseudo-starchy and sweet. The letters designate the selected progenies referred to in the text.

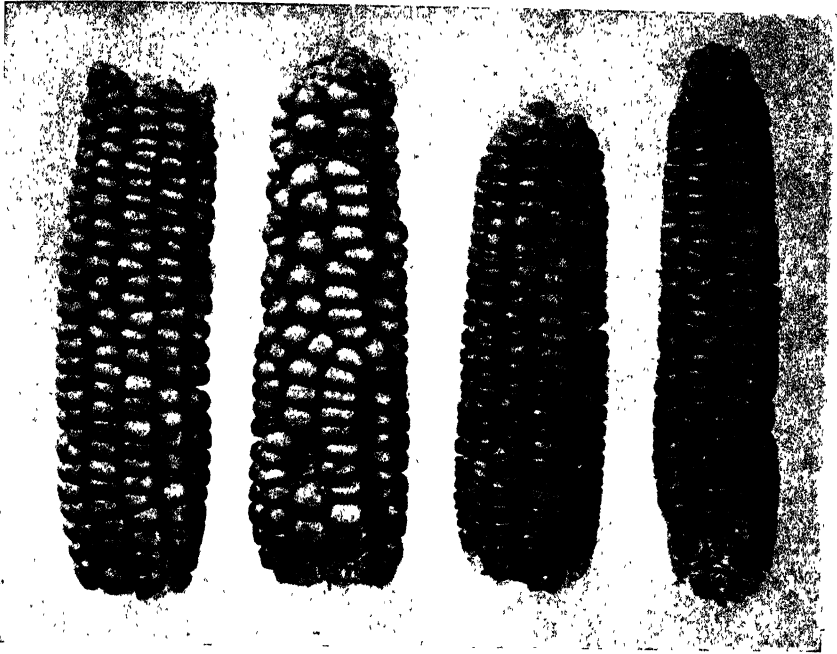


FIGURE 11.—Result of selecting the most sweet-appearing seeds from the same ear as in figure 10.

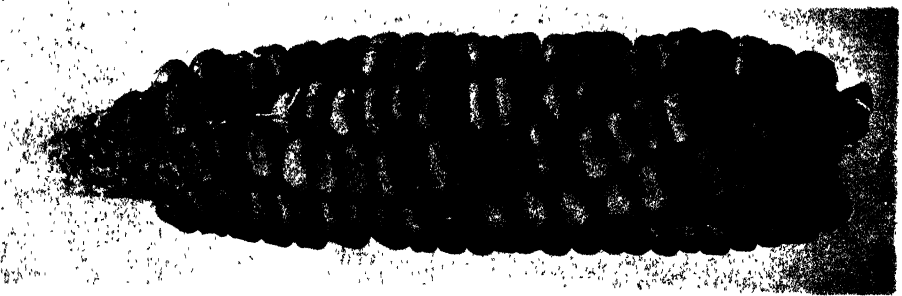


FIGURE 12.—The one ear, obtained in an F_2 plant progeny selected for starchiness, which is segregating in a mono-hybrid ratio into opaque and translucent seeds.

altering the appearance of the seeds. The F_1 ears, A, are intermediate with some variability between different ears, which is assumed to be non-genetic, and show indefinite segregation in the seed populations. Selection of extreme seeds on one of these ears gives F_2 progenies, B and C, which are still intermediate and show some response in the direction of selection although the differences between the two progenies are small. Individual ear selections from these two F_2 progenies give F_3 progenies, D and I, which show a marked response to selection. In the starchy progeny, D, ten self-pollinated ears were obtained which altogether resemble the parent type closely, five of these ears being apparently as pure for the character as any ears of the parental line. No ears were obtained which indicated any marked segregation among the seeds. In the sweet progeny, I, eight ears were selfed all of which show some traces of opaqueness. One or two of these ears, nevertheless, might pass as pure sweet. Some show considerable evidence of segregation. Most of them are uniform and nearly pure sweet. The differences between the two lots, D and I, are almost as great as between the two original types which went into the cross.

The two oppositely selected progenies, E and F, from the starchy selection of the previous year are nearly alike but both together are considerably more starchy than the F_2 ear from which they both came. A similar result was secured from the other two selections, G and H, coming from one ear of the sweet line. Both are almost exactly alike and together are more sweet than the F_2 ear which produced them. Nine selfed ears in each of the E and F progenies are on hand and some of each of these are visibly pure starchy. Eight and eleven ears from the two progenies, G and H, were self-pollinated and in each of these a few ears are visibly pure sweet.

Summing up these results there is proof that segregation of genetic factors has taken place in producing the F_2 seed populations grown on F_1 plants. Selection here, however, is not followed by an immediate response in the next generation although there is a slight effect, but in the following generation there is a marked response whether in plant-selected or seed-selected progenies and whether selection was in the same or in the reverse direction. In general selection is followed by a mass action which is as different from the usual types of Mendelian segregation as could be conceived. The behavior resembles that of quantitative characters governed by several factors with overlapping variability because variation is continuous. It differs from the usual cases of this kind in

that the changes initiated by selection in the F_2 seed generation do not make their appearance until two generations later. Moreover these changes cannot be checked in one succeeding generation by selecting from seed populations in the reverse direction. In this respect the behavior is unique.

The experiment outlined in the preceding diagram was duplicated with a reciprocal cross with the exception that selections from F_2 seed populations were not made, therefore the F_3 progenies, E, F, G, and H, were not grown. The two selected progenies, D and I, were grown with the same result as in the previous experiment with one notable exception in the starchy line.

In the sweet line eight ears were self-pollinated. A few are nearly pure sweet while some show slight amounts of opaqueness, some of these having indications of segregation. In the starchy line twelve ears were obtained. Eleven of these duplicate the similar ears in the first experiment. All of the ears are uniform and very nearly pure starchy. Some are entirely opaque and smooth with no indications of segregation. The remaining ear, however, shows clear-cut segregation into opaque and translucent seeds with a ratio of 3 to 1 (actual numbers 159 to 52). No other ear like this has been found. Contrary to the usual behavior segregation in this one instance is quite distinct. This ear is shown in figure 12. The first supposition is, naturally, that it resulted from an accidental out-cross with a true starchy plant the year previous. A number of facts make this possibility highly improbable. In size and conformation this exceptional ear closely resembles the eleven other ears of the same lot. Also in color of cob and seeds and in shape, size and texture of the opaque seeds there are no differences. Since the type of plant in this material is quite different from any other grown at the same time it is inconceivable that this individual could have been crossed without marked changes. Moreover the plants are reduced by inbreeding, this particular one in question being the third generation of a union of two related types, so that any out-crossing with a true starchy plant would noticeably increase the size of the plant and the ear. One such crossed plant was found in another progeny. This plant was nearly twice as tall and the ear three times as large as others in the same line and striking changes in plant and ear characters served to distinguish this individual at once. The exceptional ear under consideration was not noticed in the field at time of harvesting as starchy-sweet segregations are not clearly brought out until the seeds are dry. If the plant had shown any striking

differences, however, this would have been noted. There is no doubt in the mind of the writer but that this singular ear is a legitimate member of the family and must be considered at its face value. Moreover, the recessive segregates on this inflorescence are not strongly wrinkled as can be seen. In fact there is a considerable difference between these seeds and those of the sweet selection. The feature that makes them stand out is their translucence as contrasted with the opaqueness of the other seeds. It is therefore reasonable to assume that these sweet-appearing individuals are the result of a different factorial situation than is characteristic for other sweet varieties.

Before taking up a theoretical discussion of these results one more phase of the experiment must be considered. The cross of pseudo-starchy and true starchy maize has already been mentioned as giving clear segregation of starchy and sweet seeds in the F_2 seed populations. The sweet segregates were stated to have somewhat more opaque substances than similar segregates of the sweet-starchy cross. The two lots of sweet segregated seeds from one ear of each of the two crosses were grown with the result that the ears coming from the cross of which pseudo-starchy was one parent, were considerably more opaque than those from the other cross. No completely pseudo-starchy ears were obtained but one selfed ear was found which showed more evidences of definite segregation than any secured previously. The seeds ranged from those almost entirely smooth and opaque to those which were quite wrinkled and translucent with gradations in between. The numbers of the two kinds of seeds seemed about equal. Selection of extremes was made and the two resulting lots grown from these seeds and self-pollinated are shown—the starchy selection in figure 13 and the sweet selection in figure 14. From the selected wrinkled, translucent seeds nine selfed ears altogether were self-pollinated (three of these are not shown in the illustration as they were nearly destroyed by mold). Of these two are completely wrinkled and translucent, five show some opaqueness but are uniform and two are clearly segregating. One of these latter two (the ear at the right in figure 14) reproduces very well the parent ear from which the selections were made.

From the selected smooth, opaque seeds six selfed ears were harvested. Of these one is quite smooth and opaque, three are intermediate and uniform and two are intermediate with considerable evidence of segregation.

These results can be summed up briefly. In one selection the ears range from the selected parent type to a half-way point. In the opposite

selection the variation goes from the half-way point to the other parent. In this respect they differ from the preceding effects of selection in the pseudo-starchy by sweet material. Segregation is more pronounced and selection is followed by an immediate response. How all these facts can be most simply interpreted will be taken up in the next section.

A large number of crosses and back-crosses between the pseudo-starchy type and commercial varieties of sweet maize were made, but since all of these varieties carried more or less of the pseudo-starchy character and were in a heterozygous condition for many factors, no satisfactory analysis of the results can be made. In general the pseudo-starchy character behaved in these crosses similarly to the crosses already described.

THEORETICAL CONSIDERATION OF THE RESULTS

As far as the chemical nature of this visibly starchy endosperm is concerned the use of the term pseudo-starchy is perhaps not justified. The term was suggested by its genetic behavior largely for want of a better word which would emphasize the distinction between characters which are visibly alike but genetically so different. The term semi-starchy is undesirable because it implies a relation between the new endosperm condition and the well known starchy endosperm where no relationship apparently exists.

Since reciprocal pollinations between pseudo-starchy and sweet have no marked effect the character differs in its mode of inheritance from ordinary endosperm characters where dominance is displayed and distinct segregation takes place in the next generation. On the other hand it is not entirely a plant character since segregation does occur in F_2 , because selection made here is somewhat effective. It was thought at first that the character was similar to the corneous-floury endosperm character of starchy maize where (as worked out by HAYES and EAST 1915) the double condition of the female endosperm nucleus outweighs the effect of the second male nucleus and reciprocal crosses do not produce any immediate effect. In the next generation segregation is in the ratio of 1 to 1 which is merely the female gametic ratio, the second male nucleus, whatever it carries, having no power of expressing itself. The hypothesis of one such factor alone cannot be used to account for these results, particularly since it would offer no explanation for the one ear which was finally obtained where segregation is well defined and in the ratio of 3 to 1.

The simplest hypothesis that brings all the facts into line assumes both

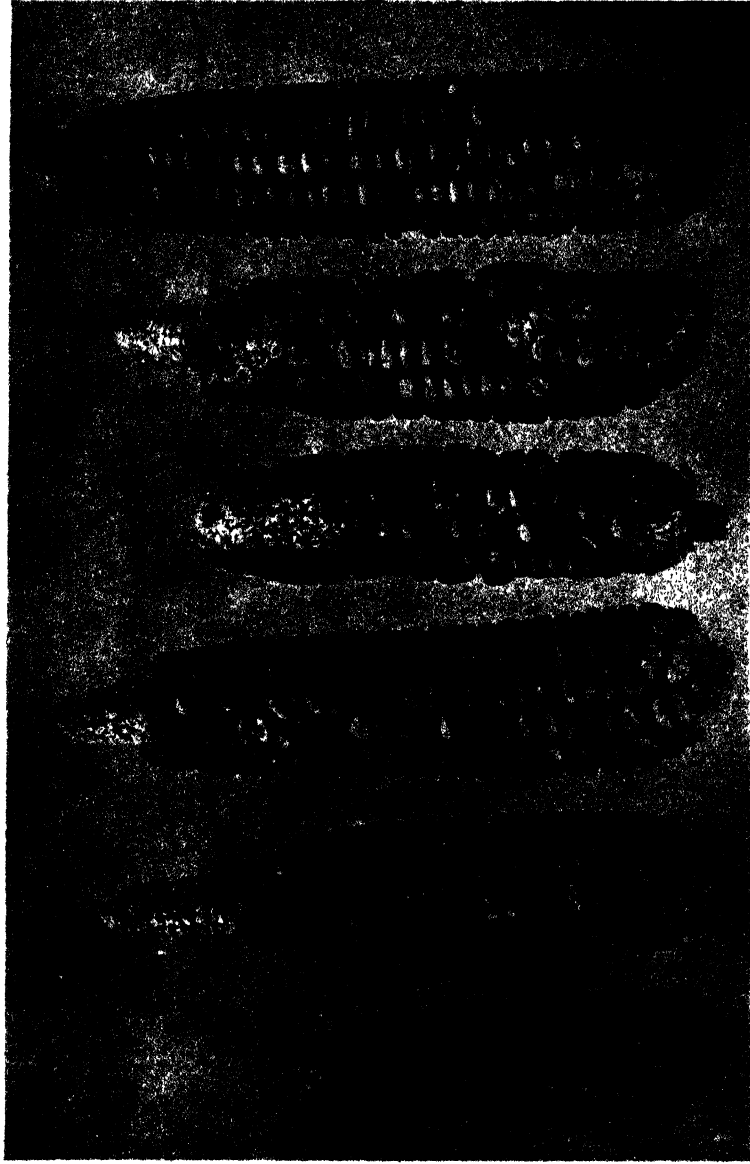


FIGURE 13.—The result of selecting the most starchy-appearing seeds from a segregating ear obtained in an extracted sweet F_2 plant progeny from a cross of pseudo-starchy by true starchy.

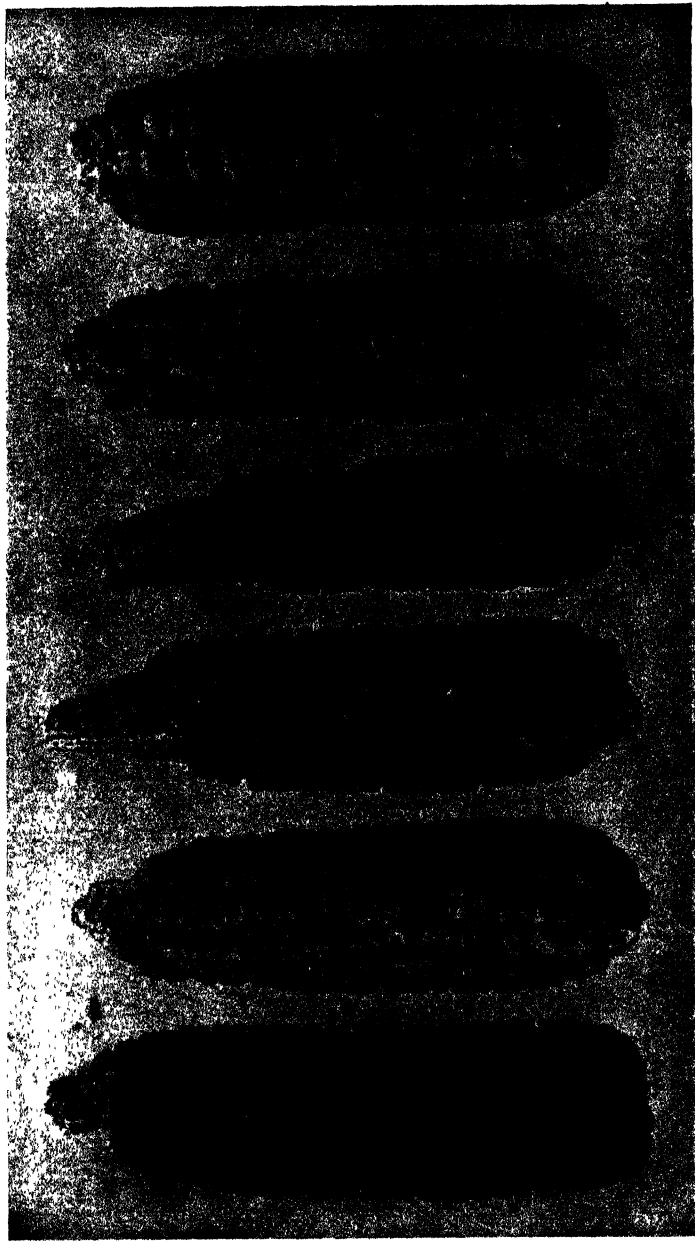


FIGURE 14.—The result of selecting the most sweet-appearing seeds from the same source as in figure 13. This parent ear is closely reproduced in the one shown at the right.

seed factors and plant factors which govern the development of this endosperm character called pseudo-starchy. The employment of plant factors which determine endosperm characters has not previously been necessary. It seems perfectly plausible, however, for an endosperm character to be dependent in some degree upon plant conditions. For example, a certain chemical substance may be needed to bring about a given result but if the plant does not supply the material the effect cannot be produced whether the immediate factor determining its production is there or not. Furthermore, we have seen that when pseudo-starchy endosperm is out-crossed to a large-seeded dent variety the resulting alteration of the shape and size of the seed and other heterotic effects governed by plant factors almost completely obliterates the expression of the pseudo-starchy character. Many factors in the maize plant determine the size and shape of the seeds; the arrangement of the corneous and floury starch, the thickness and consistency of the pericarp, the arrangement and size of the ears, so that it is quite permissible to assume that the character under investigation is modified by many plant factors which have no direct relation to endosperm factors themselves.

In addition to plant determiners there must be at least one endosperm factor difference which, with a suitable basic factorial composition, determines the production of opaqueness or translucence in the seeds. The evidence for this is based on only one segregating ear but it is difficult to avoid this assumption. The visible effect of this gene, in a proper medium, is much the same as the one governing the well known difference between starchy and sweet varieties of maize. In both cases dominance is complete and segregation is distinct in a mono-hybrid ratio.

Besides this one endosperm factor which brings about usual Mendelian segregation, there is evidence for another of the same nature as the one concerned in the corneous-floury condition of maize, perhaps the very same factor, only here acting in the absence of true starchiness. The part which this gene plays follows the female gamete in its visible effect, i.e., the double dose in the fusion nucleus almost entirely overcomes the single dose coming from the second male nucleus and segregation is in an equal ratio. The evidence for this assumption is based on the segregating ear and the two selected progenies obtained from it shown in figures 13 and 14.

As to the exact number of plant factors involved in the material worked with the rather scanty data and the indefinite nature of the character do not permit any reliable analysis. As an illustration it will be

sufficient to assume one such factor of such a nature that without it the two endosperm determiners cannot produce the full expression of the pseudo-starchy character. These three factors, each different in mode of operation, but all working towards the same end, may be used arbitrarily to show what can be expected from such a scheme and I believe they will satisfy all reasonable demands as they are used to show merely how recombination of such Mendelian units *may* account for the results. This can be done without a thorough analysis of all the factors concerned. The three factors assumed are as follows:

A—a plant factor necessary for the full expression of pseudo-starchiness in the endosperm, which part of the organism is one generation beyond the plant in which this factor operates.

B—an endosperm factor which prevents the characteristic shrinking of sweet seeds; this factor has a greater effect when coming from the female side than from the male, i.e., *BBb* and *bbB* appear different but behave genetically exactly alike.

C—an endosperm factor determining opaqueness, which shows dominance, i.e., *CC* and *Cc* are visibly alike. *C* and *c* have their greatest differentiating effect only when *A* and *B* are present in the homozygous state.

It is also assumed that *A* and *B* are cumulative in effect, whereas *C* is not. According to this scheme:

Plant	Seed
<i>AA</i>	<i>BBB CC</i> = pseudo-starchy endosperm;
<i>aa</i>	<i>bbb cc</i> = sweet endosperm.

The reciprocal pollinations are without effect because of the following factorial situation:

Plant	Seed
<i>AA</i>	<i>BBb Cc</i> = pseudo-starchy × sweet;
<i>aa</i>	<i>bbB cC</i> = sweet × pseudo-starchy.

In the first cross the double dose of *B* and the dominating nature of *C* prevent any noticeable wrinkled or translucent effects appearing. In the reciprocal pollination the single doses of *B* and *C* are non-operative, or feebly so, in the absence of *A*. (*A*, of course, is brought into the seed, but has nothing to do with the plant.) In the following generation which is the F_1 for the plant and F_2 for the seed, the situation set forth in table 3 can be expected:

TABLE 3

The theoretical composition and the appearance of F_2 seeds with respect to the endosperm factors B and C and their behavior in the following generation according to the plant factor A .

Composition of F_1 plant	Composition and number of F_2 seeds	Appearance of F_2 seeds	Appearance of F_2 ears grown from F_2 seeds according to the composition of the plant with respect to AA , Aa or aa
Aa	1 $BBB CC$ 4	Smooth, opaque	Intermediate, uniform except with AA which gives pure pseudo-starchy.
	2 $BBB Cc$ 8	Smooth, opaque	Intermediate, variable except with AA which segregates 3 to 1.
	1 $BBb CC$ 4	Smooth, opaque	Intermediate, variable
	2 $BBb Cc$ 8	Smooth, opaque	Intermediate, variable
	1 $bbB CC$ 4	Wrinkled, opaque	Intermediate, variable
	2 $bbB Cc$ 8	Wrinkled, opaque	Intermediate, variable
	1 $BBB cc$ 4	Smooth, translucent	Intermediate, uniform
	1 $BBb cc$ 4	Smooth, translucent	Intermediate, variable
	1 $bbB cc$ 4	Wrinkled, translucent	Intermediate, variable
	1 $bbb CC$ 4	Wrinkled, translucent	Intermediate, uniform except with aa which gives sweet appearance.
	2 $bbb Cc$ 8	Wrinkled, translucent	Intermediate, variable except with aa which gives sweet appearance.
	1 $bbb cc$ 4	Wrinkled, translucent	Intermediate, uniform except with aa which gives pure sweet.
	— 16	— 64	

The F_2 seeds fall into four theoretical groups:

Smooth, opaque	6
Wrinkled, opaque	3
Smooth, translucent	2
Wrinkled, translucent	5

—
16

But none of these groups is sharply expressed because of the heterozygous nature of the plant having Aa . Moreover there will be variability according to whether the seed has the composition BBB or BBb and bbB or bbb as the hypothesis states that B is cumulative in effect. These causes for an intermediate position with hereditary variability together

with some non-genetic fluctuation are sufficient to account for almost any amount of indefinite segregation in F_2 seed populations. Moreover there may easily be a physiological correlation between smoothness and opaqueness on the one hand and wrinkledness and translucence on the other such that the other two combinations are not perfectly developed.

The behavior of the F_2 seeds in the following generation depends not only on their composition with respect to B and C , but also upon their carrying AA , Aa or aa . None of these three combinations of this factor have anything to do with the appearances of the seeds that bear them, but they will have a marked effect upon the ears grown from these seeds. The extreme complexity of the F_2 plant population with only these three factors is readily apparent. Moreover, selection of F_2 seeds will have little efficacy in the following generation because of the difference in appearance, but similarity in behavior, of BBb and bbB together with the fact that the A or a factors do not make their presence known. Nearly all of the F_2 ears bearing F_3 seeds will be intermediate, some tending towards one type and some towards the other, but part of them will be uniform with respect to the seed population and part will be variable, i.e., will show more or less evidence of segregation. This is an important point as it is the best means of reconciling the theory with the actual results obtained.

Summing up the expectation for the F_2 plants the following numbers are obtained:

- 1 Pure pseudo-starchy, like parent, breeds true (viz., $AA BBB CC$);
- 2 Nearly pure pseudo-starchy, do not breed true (viz., $Aa BBB CC$);
- 11 Intermediate and uniform;
- 44 Intermediate and variable;
- 3 Nearly pure sweet like parent, breed true (viz., $aa bbb CC$ or Cc);
- 1 Pure sweet like parent, breeds true (viz., $aa bbb cc$);
- 2 Segregate 3 to 1 opaque-translucent (viz., $AA BBB Cc$).

64.

Without going into all the possibilities of this factorial arrangement, the actual results fit in reasonably well although it is granted that the theoretical expectations are sufficiently complicated to cover almost any variable character like this one. Several agreements of facts with theory justify this interpretation tentatively without analyzing this situation completely. Only about ten self-fertilized ears were obtained in each generation in a progeny. Selection of F_2 seeds was followed by very slight response in the next generation. According to the theory 55 out

of 64 are expected to be intermediate in various degrees, but the expression of the pseudo-starchy character in the F_2 ear is not closely correlated with the appearance of the F_2 seed which produced it. Selection of F_2 plants however is expected to have a marked effect on the following generation because the extreme ears are homozygous for one or more factors for starchiness or sweetness as the case may be. Moreover the appearance of the ears as a whole in this generation is more closely associated with the genetical composition of the plant so that plant selection is effective.

The segregating F_3 seed populations are like segregating F_2 populations with respect to endosperm factors and seed selection is followed by as little response in this generation as in the preceding. The fact that the two reversely selected progenies in F_3 (F and G in the diagram page 378) differ greatly, can be attributed to the possibility that in the starchy-selected line the segregating ear was homozygous for AA and that in the sweet-selected line a similar appearing ear was homozygous for aa . Such an occurrence would be somewhat of a coincidence, it is true, but one that could easily happen.

Some of the F_2 ears obtained looked like pseudo-starchy, but two which were tested further did not prove to be a complete return to the pseudo-starchy parent. Nor is this remarkable in view of the small numbers, since only one out of 64 is expected. According to theory it is somewhat easier to recover a true-breeding sweet. None of the F_2 ears approached the sweet parent as closely as the extremes in the opposite direction resembled the other parent, and in F_3 neither of the two sweet-selected progenies duplicated the sweet parent but several individual ears look like pure sweet. By far the majority of the ears obtained are intermediate. Some of these are uniform and some are variable, but it is impossible to classify them numerically as one grades into the other so indistinctly.

No definitely segregating ear appeared in F_2 and only one in F_3 in a total of 22 ears in two separate lines selected for starchiness. In an unselected population such ears would be expected once in 32 times. According to the hypothesis these segregating ears occur only when A and B are present and homozygous. Perhaps it would be more reasonable to expect them when A is heterozygous as well as homozygous. In that case such an ear would be looked for 6 in 64 times in an unselected population and more frequently in a starchy selection. On this assumption, only one ear was found where several should have appeared.

Let us now consider the other selection experiment, starting with a

variable ear which, it will be remembered, came from the extracted sweet seeds of a cross of pseudo-starchy by true starchy. Since segregation is more distinct on this ear than upon any ear in the F_2 seed generation of the other cross, the logical supposition is that there are less genetical differences involved in this case. In other words, let us assume that the true starchy variety used in making the cross also possessed some of the factors for pseudo-starchiness as well as the main factor for true starchiness. There is evidence to support this assumption from the fact that other crosses, not specifically mentioned previously, of sweet \times true starchy, showed traces of pseudo-starchiness in the sweet-segregating seeds.

From our scanty data there is no way of knowing how many factors were brought in by the starchy parent. To fit the interpretation to the preceding discussion it will be assumed that there has been a simplification of the genetical composition such that the segregating ear in question is homozygous for the C factor and heterozygous for only A and B . The C factor difference is the one ruled out because no evidence was obtained in this material of a clean-cut segregation approximating 3 to 1 as in the previous case.

The ear used in this second selection experiment, then, supposedly has been produced on a plant homozygous for the C factor and heterozygous for A , and having four visibly different kinds of seeds according to their composition with respect to B , as follows:

Plant	Seeds
Aa	$\left\{ \begin{array}{l} BBB \\ BBb \end{array} \right\}$ selected starchy; $\left\{ \begin{array}{l} bbB \\ bbb \end{array} \right\}$ selected sweet.

With the assumption as before, that B does not differentiate sharply, the seeds gradate from nearly pure starchy to nearly pure sweet (see figure 14, ear on the right) and the variation is equally balanced between the two extremes. C is present in all seeds but has little effect with bb and heterozygous Aa . Selection of the most starchy- and most sweet-appearing seeds consists of choosing the BBB and BBb seeds on the one hand, and the bbB and bbb seeds on the other. All four types carry indiscriminately AA , Aa or aa , so that the numerical possibilities in the next generation are as shown in table 4.

TABLE 4

The theoretical result, in the following generation, of selecting starchy- and sweet-appearing seeds from a segregating ear borne on a plant homozygous for C and heterozygous for A and B.

Type of seeds selected	Composition and number of F ₂ seed populations and F ₂ plants which produce them		Appearance of F ₂ seed populations
Starchy seeds <i>BBB</i>	8	Plant Seeds $\begin{cases} AA & BBB & 2 \\ Aa & BBB & 4 \\ aa & BBB & 2 \end{cases}$	Pure pseudo-starchy
			Intermediate, uniform
			Intermediate, uniform
Starchy seeds <i>BBb</i> and sweet seeds <i>bbB</i>	16	$\begin{cases} AA & BBB & 1 \\ AA & \begin{cases} BBB \\ BBb \\ bbB \\ bbb \end{cases} & 2 \\ AA & bbb & 1 \\ Aa & BBB & 2 \\ Aa & \begin{cases} BBB \\ BBb \\ bbB \\ bbb \end{cases} & 4 \\ Aa & bbb & 2 \\ aa & BBB & 1 \\ aa & \begin{cases} BBB \\ BBb \\ bbB \\ bbb \end{cases} & 2 \\ aa & bbb & 1 \end{cases}$	Pure pseudo-starchy
			Intermediate, variable
			Intermediate, uniform
			Intermediate, uniform
			Intermediate, variable
			Intermediate, uniform
			Intermediate, uniform
			Intermediate, variable
			Pure sweet-appearing
Sweet seeds <i>bbb</i>	8	$\begin{cases} AA & bbb & 2 \\ Aa & bbb & 4 \\ aa & bbb & 2 \end{cases}$	Intermediate, uniform
			Intermediate, uniform
			Pure sweet-appearing
	32	32	

Summing up the theoretical situation and comparing this with the results actually obtained, the agreement is close.

TABLE 5

Numbers found	Classification of ears in both selected progenies	Proportion	
		Expected	Found
1	Pure starchy	3	2
8	Intermediate and uniform	18	16
4	Intermediate and variable	8	8
2	Sweet-appearing	3	4
15		32	30

None of the apparently pure types have been grown as yet to test the propriety of their classification. The intermediate ears, both uniform and variable, differ in the degree in which they approach the one or the other extreme as shown in figures 13 and 14. The distinction between intermediate variable and intermediate uniform is somewhat arbitrary although it can be easily seen in the illustration that some of the ears are segregating and others are not.

It should be noted that, according to theory, the sweet ears of this last material have a different constitution than the sweet segregated seeds of the one notable ear of the other material. In that segregating ear the translucent seeds are given the formula $AA\ BBB\ cc$ while in this case the sweet seeds are $aa\ bbb\ CC$. The seeds of both are translucent. In the latter they are strongly wrinkled while in the former they are not. Whether or not these assumptions are correct can be easily tested by crossing the two different types of sweet seeds. The pollination made one way should give pseudo-starchy seeds,—made the other way should have very little effect, as the reciprocally crossed seeds differ markedly in composition. In the first instance they are $AA\ BBb\ cC$ pseudo-starchy; in the other $aa\ bbB\ Cc$ sweet or only slightly modified. In the next generation both crosses should give intermediate, indefinitely segregating ears similar to the ones shown in figure 9, with the possibility of recovering complete pseudo-starchiness in later generations.

CONCLUSION

The reader may question the desirability of presenting such an elaborate hypothesis with so few data to support it. The inheritance of pseudo-starchy endosperm is a task in itself and is not primarily the problem we undertook to solve in determining the effects of selection. The inheritance of this character will be followed up. It will be surprising if the interpretation given here will be entirely adequate to cover all the possibilities in this material. The one presented shows how a

complicated situation may be due to relatively simple genetical differences. And whether or not the interpretation given is wholly correct is aside from the main point at issue since it seems clear to the writer that some similar factorial analysis will bring the results into alignment if the one given is not altogether satisfactory.

What we undertook to find out was:

(1) Whether or not this original semi-starchy condition was an exception to the complete segregation of the two allelomorphs represented in starchy and sweet endosperms.

(2) Whether or not the effect of selection upon this apparent blend was the result of a progressive change of a single unstabilized Mendelian unit.

Both of these questions can be answered decidedly in the negative. It has been shown beyond doubt that pseudo-starchiness, as described here, and true starchiness, as commonly understood, in maize endosperm, are genetically dissimilar. Therefore, segregation of the one principal allelomorphic pair involved in the original starchy and sweet endosperm types was complete. It has also been clearly shown that several Mendelian units are concerned with this new character and that therefore the effect of selection upon this material, in all probability, is due to the sorting-out and re-arrangement of various of these units. The evidence for this lies in the facts that (1) selection was most effective in the early generations of self-fertilization and alterations ceased as homozygosis in other factors was brought about; (2) when the extremes of selection were crossed no clear-cut segregations occurred in F_2 but in later generations, as the germplasm was automatically simplified by self-fertilization, different types of segregation of a more definite nature appeared, with which selection was more effective.

To believe in the truth of these assertions it is not necessary to have the inheritance of all factors having to do with this rather complex endosperm situation worked out in all detail. Such a line of investigation is interesting but less important than the main points already covered. This material is not favorable for making an exact and minute genetical analysis. For this purpose we already have at hand a number of beautiful illustrations which are apparently far more complicated than this situation in maize but have been put on a satisfactory factorial basis. One does not hope to offer anything from this endosperm character, equal, for example, to the rigorous analysis of the beaded wing character in *Drosophila* by MULLER (1918). The recent work of EMERSON (1918)

with aleurone color and of LINDSTROM (1918) with chlorophyll color in maize ought also to be noted in this connection. Our meager results considered with such investigations as these and with other experiments dealing directly with the problem of selection, particularly those of MAC DOWELL (1915), PEARL (1917), MORGAN (1917), STURTEVANT (1918) and PAYNE (1918) fit in with the general conclusion to be derived from present-day genetical research along this line, which is, that the hypothesis of stable hereditary units, while similarly subject to limitations, is as useful in the field of genetics as the atomic theory is in the realm of chemistry.

SUMMARY

1. Amylaceous seeds in an extracted recessive progeny from a cross of starchy and sweet varieties of maize suggested that an imperfect segregation or contamination of the two allelomorphs might have occurred. This material was the basis for a selection experiment attempting to recover both parental types.

2. After five generations of selection in opposite directions in self-fertilized lines apparently pure sweet and pure starchy strains were obtained which remained constant during from four to five additional years of self-fertilization and selection.

3. When crossed with an ordinary variety of starchy maize both the sweet- and starchy-appearing selections behave the same, segregating 3 to 1 in the second generation into starchy and sweet seeds.

4. Examination of the starch grains of the two selections shows them to be much alike and both lots together are not fully developed as compared to starchy varieties.

5. In chemical composition the starchy selection is more like other starchy types, particularly in soluble and insoluble carbohydrates.

6. Nevertheless, on account of its behavior in inheritance, from the nature of its starch grains and the qualities of the seeds this starchy-appearing selection is considered to be independent and genetically distinct from true starchiness. This new form of endosperm is called pseudo-starchy.

7. Crosses of pseudo-starchy and sweet give indistinctly segregating seed populations in F_2 which show little response to selection in the next generation but in later filial periods selection is able to recover the parental patterns.

8. The pseudo-starchy type is also recovered from the extracted sweet seeds of the cross of pseudo-starchy and true starchy.

9. In both these crosses segregation becomes more distinct in later generations after the plants have been inbred. Two different kinds of segregation are observed; one giving a sharp mono-hybrid ratio of opaque and translucent seeds; the other giving a less definite splitting in a 1 to 1 fashion which responds immediately to selection.

10. Endosperm and plant factors working together are assumed in the interpretation of these results such that reciprocal crosses have slight immediate effect and in the F_2 seed populations the appearance of the seeds is not closely correlated with their genetic composition and hence their behavior in later generations. Tentative factorial schemes are used to illustrate ways by which these conditions may be brought about.

11. No evidence is found that imperfect segregation or contamination of factors occurred in the separation of the determiners of starchy and sweet endosperms in the original cross. The effects of selection with this character can be most logically attributed to the sorting-out and re-arrangement of hereditary factors.

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ON THE RELATIONS BETWEEN BLOOD COLOR AND COCOON COLOR IN SILKWORMS, WITH SPECIAL REFERENCE TO MENDEL'S LAW OF HEREDITY

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INTRODUCTION

A constant color correlation between cocoon and blood color is universally believed to be a fact; the yellow-cocoon-spinners are always yellow-blooded, while the white-cocoon-spinners are white-blooded. The blood color is visible through the cuticular coat of the caterpillar, especially on the ventral side of the body and on the abdominal legs; so that, by observing these, we were led to believe that we could tell exactly of what color the cocoon would be. Even in the elaborate studies of my revered teacher, the late Prof. Dr. TOYAMA, and Mr. TANAKA, on the inheritance of cocoon color in the silkworm, the cocoon color was mostly determined from the blood color during the larval stage, for fear of losing worms incidental to rearing.

To my great surprise, some white cocoons were accidentally reared from a batch which contained yellow-blooded caterpillars only, in the autumn of 1916. At first it was thought that they had been taken by mistake from other batches; but the yellow color of the blood seen through the bodies of those pupae cleared away that doubt. All the individuals of the next generation, paired among those yellow-blooded white-cocoon-spinners, displayed the same character as their parents, i.e., the

yellow-blooded worms produced white cocoons,—this must be a new discovery.

I have ascertained that they are pure bred so far as the color of blood and cocoon is concerned, having bred seven generations of this new race since then.

From this fact it can be positively said that the yellow-blooded silkworms do not necessarily spin yellow cocoons. To investigate the relation between the blood color and the cocoon color in silkworms I began experiments in the spring of 1917, which are not yet completed. Part of my work, however, is now accomplished and the following series of experiments will throw some light on it.

Before giving details of my investigations, I wish to express my heart-felt thanks to Dr. TATSUSHIRŌ KAGAYAMA, the Director of the STATION, and Prof. Dr. CHIYOMATSU ISHIKAWA, for their valuable advice and their kindness in revising the manuscript.

MATERIALS, METHODS AND REMARKS

For brevity's sake I make use of the following abbreviations:

YY refers to yellow-blooded yellow-cocoon-spinners, and in some cases to the character itself, too.

YW refers to yellow-blooded white-cocoon-spinners, and in some cases to the character itself, too.

WW refers to white-blooded white-cocoon-spinners, and in some cases to the character itself, too.

I selected the following seven races as materials for hybridizing with the yellow-blooded white-cocoon-spinner, which, as already mentioned, was newly discovered in our INSTITUTE.

“Onodahime”, a Japanese tetra-voltine yellow race which bred true to YY for 32 generations;

“Seiyō”, a Chinese di-voltine yellow race which bred true to YY for 6 generations.

“Tōbuhime”, a Japanese di-voltine white race which bred true to WW for 14 generations.

“Shōzan”	} Chinese di-voltine white races which bred true to WW for 7 or 8 generations.
“Simpaku”	
“Jōsai”	
“Shōkō”	

I adopted the artificial methods of hatching the eggs and thus secured eight generations in two years. I pursued three methods of hatching, (a) immersion in hot water, (b) immersion in dilute or concentrated

hydrochloric acid and (c), by electricity. Of these three I can not say which was the best, for I obtained quite good results with each of them; but it is certain that the worms were a little weaker than those naturally hatched and the percentage of worms lost by disease and through mistakes while raising, was 3.5 percent to 79.6 percent.

As to the method of breeding there is nothing particular to say; but it may be mentioned here that the worms from each parent were reared in a separate basket, so that each lot number in the tables in this paper comprises eggs laid by a single parent moth.

CROSSES BETWEEN YW AND YY

F₁ generation

Reciprocal crosses were made and 2584 *F₁* cocoons were reared. All were YY, and I was unable to detect any difference between the pure YY parent and the *F₁* hybrids, in the intensity of the yellow color of their cocoons. We may conclude, therefore, that YY is perfectly dominant over YW.

F₂ generation

The *F₂* generation produced by the *F₁* hybrids above described were found to contain YY and YW in the ratios shown in table I.

TABLE I

Lot No.	<i>F₂</i> generation			
	YY	YW	Totals	YY : YW per 4
A-'17- 956	178	55	233	3.056 : 0.944
A-'17- 957	184	55	239	3.079 : 0.921
A-'17- 959	182	53	235	3.098 : 0.902
A-'17-1099	63	20	83	3.036 : 0.964
A-'17-1101	180	68	248	2.903 : 1.097
A-'17-1102	165	64	229	2.882 : 1.118
A-'18- 238	187	51	238	3.143 : 0.857
A-'18- 239	220	69	289	3.045 : 0.955
A'-'17- 965	170	74	244	2.787 : 1.213
A-'17- 966	211	76	287	2.941 : 1.059
A-'17- 967	163	48	211	3.090 : 0.910
Total	1903	633	2536	3.002 : 0.998

$$a = \pm 0.002$$

$$E_m = \pm 0.023^2$$

¹ A' is the reciprocal hybrid of A. Hereafter in every table *x* and *x'*, in general, means the same relation as A to A'.

² *a* = deviation from expectation; *E_m* = probable error of the mean,

$$= \frac{0.6745 \times \text{standard deviation}}{\sqrt{\text{number of individuals}}}, \text{ i.e., } \frac{T_{\sigma}}{\sqrt{n}}.$$

Thus, it is seen that in F_2 both of the reciprocal hybrids segregated in the ratio $3YY:1YW$.

F_3 generation

It was found that YW in the former generation produced 1127 YW offspring without a single exception in the six batches raised.

Some of the YY in F_2 produced exclusively YY offspring and the others were found to segregate in the ratio $3YY:1YW$, as shown in table 2.

But the ratio between those which bred true to type in F_3 , and those which segregated in the ratio $3:1$, can not be readily ascertained because of their inability to self-fertilize.

TABLE 2

Lot No.	Character of F_2	F_2 generation			
		YY	YW	Totals	YY : YW per 4
B ₁ -18-45	YY	192	—	192	} All YY
B ₁ -18-46	YY	172	—	172	
B ₁ -18-212	YY	68	—	68	
B ₁ -18-214	YY	244	—	244	
Total		676	—	676	
B ₂ -18-47	YY	154	52	206	2.990 : 1.010
B ₂ -18-48	YY	111	43	154	2.883 : 1.117
B ₂ -18-206	YY	64	22	86	2.977 : 1.023
B ₂ -18-207	YY	83	27	110	3.018 : 0.982
B ₂ -18-211	YY	160	62	222	2.883 : 1.117
Total		572	206	778	2.941 : 1.059

$$a = \pm 0.059$$

$$E_m = \pm 0.042$$

$$a/E_m = 1.405$$

Back crosses

I back-crossed the cross-bred form with both of the pure parent breeds, and found $F_1 \times YY$ and its reciprocal produced 516 YY only.

In the case of $F_1 \times YW$ (C) and its reciprocal (C') a segregation occurred in the ratio $1YY:1YW$ as table 3 shows.

TABLE 3

Lot No.	YY	YW	Totals	YY : YW per 4
C-17-1103	73	60	133	2.195 : 1.805
C-17-1104	110	101	211	2.085 : 1.915
C'-17-1105	22	31	53	1.660 : 2.340
C'-17-1106	64	75	139	1.842 : 2.158
C'-17-1121	30	33	63	1.905 : 2.095
Total	299	300	599	1.997 : 2.003

$$a = \pm 0.003$$

$$E_m = \pm 0.055$$

CROSSES BETWEEN YW AND WW

Although the WW used here in my experiment are pure in both blood and cocoon, being white in color, our experiments revealed three different results.

The following series of experiments show these distinctly.

CASE I

F₁ generation

All 3372 *F₁*'s produced from reciprocal ways were YY. We could not detect any visible variation in the intensity of the yellow color in the cocoons of YY *F₁*'s; more than this they took after the pure bred YY. Therefore a new character YY appeared on crossing YW with WW.

F₂ generation

As table 4 shows, in the *F₂* generation we had YY, YW and WW in the ratio of 9 : 3 : 4.

TABLE 4

Lot. No.	<i>F₂</i> generation				
	YY	YW	WW	Totals	YY : YW : WW per 16
D-'17-924	114	41	60	215	8.484 : 3.051 : 4.465
D-'17-925	152	41	72	265	9.177 : 2.475 : 4.347
D-'18-172	84	31	42	157	8.561 : 3.159 : 4.280
D-'18-173	155	54	68	277	8.953 : 3.119 : 3.928
D-'18-174	130	32	70	232	8.966 : 2.207 : 4.828
D-'18-175	213	73	89	375	9.088 : 3.115 : 3.797
D-'18-176	195	61	82	338	9.231 : 2.888 : 3.882
D-'18-178	126	50	54	230	8.765 : 3.478 : 3.757
D-'18-179	104	27	44	175	9.509 : 2.469 : 4.023
D-'18-604	80	38	41	159	8.050 : 3.824 : 4.126
D-'18-605	118	33	42	193	9.782 : 2.736 : 3.482
D-'17-940	97	21	41	159	9.761 : 2.113 : 4.126
D-'17-941	99	37	53	189	8.381 : 3.132 : 4.483
D-'17-942	123	46	58	227	8.670 : 3.242 : 4.088
D-'18-192	88	30	39	157	8.968 : 3.057 : 3.975
D-'18-606	66	28	31	125	8.448 : 3.584 : 3.968
Total	1944	643	886	3473	8.956 : 2.962 : 4.082

$$a(YY) = -0.044 \quad E_m(YY) = \pm 0.091^s$$

$$a(YW) = -0.038 \quad E_m(YW) = \pm 0.071$$

$$a(WW) = +0.082 \quad E_m(WW) = \pm 0.079 \quad a(WW)/E_m(WW) = 1.038$$

^s*a*(YY), and *E_m*(YY) mean, respectively, the deviation and the probable error in the YY group. The other symbols bear corresponding relations to the groups designated.

F₃ generation

WW in the former generation bred true to its type, producing 1277 WW in four batches; while in both YY and YW, some segregated in the ratios shown in table 5.

TABLE 5

Lot No.	Charac- ter of F ₂	F ₃ generation				
		YY	YW	WW	Totals	Ratios
E ₁ '18- 5	YY	253	—	—	253	} All YY
E ₁ '18-148	YY	200	—	—	200	
E ₁ '18-151	YY	137	—	—	137	
E ₁ '18-429	YY	130	—	—	130	
Total		720	—	—	720	
E ₂ '18- 6	YY	105	38	—	143	2.937 : 1.063
E ₂ '18-152	YY	91	33	—	124	2.935 : 1.065
E ₂ '18-430	YY	131	42	—	173	3.029 : 0.971
Total		327	113	—	440	2.973 : 1.027

$$a=\pm 0.027$$

$$E_m=\pm 0.056$$

E ₃ '18- 1	YY	160	—	54	214	2.991 : 1.009
E ₃ '18- 7	YY	268	—	112	380	2.821 : 1.179
E ₃ '18-149	YY	74	—	23	97	3.052 : 0.948
Total		502	—	189	691	2.906 : 1.094

$$a=\pm 0.094$$

$$E_m=\pm 0.044$$

$$a/E_m=2.136$$

E ₄ '18- 2	YY	110	33	55	198	8.889 : 2.667 : 4.444
E ₄ '18-147	YY	93	25	33	151	9.854 : 2.649 : 3.497
E ₄ '18-150	YY	34	10	11	55	9.891 : 2.909 : 3.200
Total		237	68	99	404	9.386 : 2.693 : 3.921

$$a(YY)=+0.386$$

$$E_m(YY)=\pm 0.266$$

$$a(YY)/E_m(YY)=1.451$$

$$a(YW)=-0.307$$

$$E_m(YW)=\pm 0.210$$

$$a(YW)/E_m(YW)=1.462$$

$$a(WW)=-0.079$$

$$E_m(WW)=\pm 0.233$$

TABLE 5 (continued)

E_6 -18-431	YW	—	136	—	136	} All YW
E_5 -18-432	YW	—	203	—	203	
E_6 -18-433	YW	—	178	—	178	
Total		—	517	—	517	
E_6 -17-1024	YW	—	44	20	64	2.750 : 1.250
E_6 -18- 8	YW	—	336	88	424	3.170 : 0.830
E_6 -18- 12	YW	—	247	81	328	3.012 : 0.988
Total		—	627	189	816	3.074 : 0.926

$$a = \pm 0.074$$

$$E_m = \pm 0.041$$

$$a/E_m = 1.805$$

Thus we see that there are four types in the genetical constitution of YY, though they are alike in appearance, and two types in the YW group of the F_2 generation.

Back crosses

(a) $F_1 \times YW(F)$ and its reciprocal (F')

YY and YW occurred in the ratio of 1 : 1 as shown in table 6.

TABLE 6

Lot No.	YY	YW	Totals	YY : YW per 4
F'-17- 932	125	146	271	1.845 : 2.155
F'-17- 933	131	139	270	1.941 : 2.059
F'-17- 948	80	78	158	2.025 : 1.975
F'-17- 949	102	117	219	1.863 : 2.137
F'-17-1032	128	90	218	2.349 : 1.651
F'-17-1038	59	56	115	2.052 : 1.948
F'-18- 646	29	42	71	1.634 : 2.366
F'-17- 934	106	69	175	2.423 : 1.577
F'-17- 935	108	91	199	2.171 : 1.829
F'-17- 950	61	67	128	1.906 : 2.094
F'-17- 951	74	62	136	2.176 : 1.824
F'-17-1039	50	56	106	1.887 : 2.113
F'-17-1042	51	62	113	1.805 : 2.195
F'-17-1090	47	44	91	2.066 : 1.934
F'-18- 647	28	29	57	1.965 : 2.035
Total	1179	1148	2327	2.027 : 1.973

$$a = \pm 0.027$$

$$E_m = \pm 0.028$$

(b) $F_1 \times WW (G)$ and its reciprocal (G')

In this case YY and WW occurred in the ratio 1 : 1, as table 7 shows.

TABLE 7

Lot No.	YY	WW	Totals	YY : WW per 4
G-'17- 928	228	187	415	2.198 : 1.802
G-'17- 929	200	173	373	2.145 : 1.855
G-'17- 944	168	164	332	2.024 : 1.976
G-'17- 945	139	137	276	2.014 : 1.986
G-'17-1124	192	214	406	1.892 : 2.108
G-'18- 633	50	57	107	1.869 : 2.131
G-'18- 639	111	125	236	1.881 : 2.119
G-'18- 640	55	47	102	2.157 : 1.843
G-'18- 642	121	109	230	2.104 : 1.896
G-'18- 643	80	102	182	1.758 : 2.242
G-'18- 652	75	82	157	1.911 : 2.089
G-'18- 658	90	88	178	2.022 : 1.978
G-'17- 930	113	110	223	2.027 : 1.973
G-'17- 931	122	134	256	1.906 : 2.094
G-'17- 946	146	137	283	2.064 : 1.936
G-'17- 947	169	140	309	2.188 : 1.812
G-'17-1049	87	78	165	2.109 : 1.891
G-'17-1050	110	127	237	1.857 : 2.143
G-'18- 42	129	164	293	1.761 : 2.239
G-'18- 634	72	64	136	2.118 : 1.882
G-'18- 635	83	63	146	2.274 : 1.726
G-'18- 644	81	70	151	2.146 : 1.854
Total	2621	2572	5193	2.019 : 1.981

$$a = \pm 0.019$$

$$E_m = \pm 0.019$$

CASE II

F₁ generation

In contrast to case I, the YW in this case was entirely dominant over WW,—all the 3075 *F₁*'s made in both reciprocal ways were YW.

F₂ generation

As table 8 shows, simple Mendelian segregation occurs in the *F₂* generation.

F₃ generation

Out of seven batches of the WW group, I reared 1650 white cocoons, i.e., WW bred true to its type. But the YW group did not always do so. Out of the six batches reared two segregated in the ratio of 3YW : 1 WW, as the following table shows.

TABLE 8

Lot No.	F ₂ generation			
	YW	WW	Totals	YW : WW per 4
H-'17-1027	103	27	130	3.169 : 0.831
H-'18- 182	277	120	397	2.791 : 1.209
H-'18- 183	219	82	301	2.910 : 1.090
H-'18- 184	206	71	277	2.975 : 1.025
H-'18- 185	260	79	339	3.068 : 0.932
H-'18- 718	126	43	169	2.982 : 1.018
H-'18- 719	167	63	230	2.904 : 1.096
H-'18- 720	118	36	154	3.065 : 0.935
H-'17-1075	49	15	64	3.063 : 0.938
H-'17-1081	47	15	62	3.032 : 0.968
H-'18- 193	154	50	204	3.020 : 0.980
H-'18- 194	233	66	299	3.117 : 0.883
H-'18- 195	181	69	250	2.896 : 1.104
Total	2140	736	2876	2.976 : 1.024

$a = \pm 0.024$
 $E_m = \pm 0.022$
 $a/E_m = 1.091$

TABLE 9

Lot No.	F ₃ generation			
	YW	WW	Totals	YW : WW per 4
I ₁ -'18- 13	186	—	186	} All YW
I ₁ -'18- 14	213	—	213	
I ₁ -'18-153	102	—	102	
I ₁ -'18-449	251	—	251	
Total	752	—	752	
I ₂ -'18-446	63	20	83	3.036 : 0.964
I ₂ -'18-588	139	39	178	3.124 : 0.876
Total	202	59	261	3.096 : 0.904

$a = \pm 0.096$
 $E_m = \pm 0.067$
 $a/E_m = 1.433$

Back crosses

I back-crossed the cross-bred form with both of the pure parent breeds, and found $F_1 \times YW$ and its reciprocal produced 1958 YW only. In the case of $F_1 \times WW$ (J) and its reciprocal (J') a segregation occurred in the ratio of 1YW : 1WW as shown in table 10.

CASE 3

F₁ generation

Differing from both case 1 and case 2, YY and YW occurred in the ratio of 1 : 1 even in the F_1 generation.

TABLE 10

Lot No.	YW	WW	Totals	YW : WW per 4
J-'18-199	108	92	200	2.160 : 1.840
J-'18-712	48	37	85	2.259 : 1.741
J-'18-702	65	54	119	2.185 : 1.815
Total	221	183	404	2.188 : 1.812
$a=\pm 0.188$		$E_m=\pm 0.067$		$a/E_m=2.806$

TABLE 11

Lot No.	F_1 generation			
	YY	YW	Totals	YY : YW per 4
K-'18-240	102	114	216	1.889 : 2.111
K-'18-525	39	36	75	2.080 : 1.920
K-'18-527	28	36	64	1.750 : 2.250
K-'18-528	46	35	81	2.272 : 1.728
K-'18-550	129	105	234	2.205 : 1.795
K-'18-569	137	164	301	1.821 : 2.179
K-'17-523	23	18	41	2.244 : 1.756
K-'18-490	64	63	127	2.016 : 1.984
K-'18-553	164	155	319	2.056 : 1.944
K-'18-570	108	118	226	1.912 : 2.088
Total	840	844	1684	1.995 : 2.005
$a=\pm 0.005$		$E_m=\pm 0.033$		

 F_2 generation

YY and YW as in the F_1 , segregated in the ratios shown in table 12.

TABLE 12

Lot No.	Character of F_1	F_2 generation				
		YY	YW	WW	Totals	Ratios
L ₁ -'18-723	YY	29	11	22	62	7.484 : 2.839 : 5.677
L ₁ -'18-708	YY	76	25	33	134	9.075 : 2.985 : 3.940
Total		105	36	55	196	8.571 : 2.939 : 4.490
$a(YY)=-0.429$		$E_m(YY)=\pm 0.382$		$a(YY)/E_m(YY)=1.123$		
$a(YW)=-0.061$		$E_m(YW)=\pm 0.301$		$a(YW)/E_m(YW)=1.467$		
$a(WW)=+0.490$		$E_m(WW)=\pm 0.334$		$a(WW)/E_m(WW)=1.467$		
L ₂ -'18-722	YW	—	141	41	182	3.099 : 0.901
L ₂ -'18-709	YW	—	75	19	94	3.191 : 0.809
Total		—	216	60	276	3.130 : 0.870
$a=\pm 0.130$		$E_m=\pm 0.070$		$a/E_m=1.857$		

Thus it is seen that case 3 showed practically the same results from the YY as in case 1 and from YW as in case 2.

Back crosses

There were two types in the F_1 generation as above mentioned, so in back-crossing the cross-bred form with both of the pure parent breeds there are four cases, as shown in the following tables.

(1) $F_1(YY) \times YW$

In this back-crossing practically the same results were obtained as in the back-crossing in case 1.

TABLE 13

Lot No.	YY	YW	Totals	Ratio
M-'18-710	71	96	167	1.701 : 2.299
M-'18-725	164	172	336	1.952 : 2.048
Total	235	268	503	1.869 : 2.131

$$a = \pm 0.131$$

$$E_m = \pm 0.060$$

$$a/E_m = 2.183$$

(2) $F_1(YW) \times YW$

In this case there were 381 YW only, without a single exception. This is quite parallel to the back-crossing in case 2.

(3) $F_1(YY) \times WW$

Here there was a very interesting result. Three different types of segregation were observed:

(a) 1YY : 1WW

(b) 1YY : 1YW : 2WW

(c) 3YY : 1YW : 4WW

Among these (a) is a case quite parallel to the back-crossing in case 1, and both (b) and (c) are newly observed ratios.

TABLE 14

Lot No.	YY	YW	WW	Totals	Ratios
N ₁ -'18- 457	105	—	120	225	1.867 : 2.133
N ₁ -'18- 458	144	—	139	283	2.035 : 1.965
N ₁ -'18- 459	163	—	154	317	2.057 : 1.943
Total	412	—	413	825	1.998 : 2.002

$$a = \pm 0.002$$

$$E_m = \pm 0.047$$

TABLE 14 (continued)

N_2 -18- 469	25	31	68	124	0.806 : 1.000 : 1.194
N_2 -18- 470	24	34	45	103	0.932 : 1.320 : 1.748
Total	49	65	113	227	0.863 : 1.145 : 1.991
$a(YY) = -0.137$ $E_m(YY) = \pm 0.078$ $a(YY)/E_m(YY) = 1.756$ $a(YW) = +0.145$ $E_m(YW) = \pm 0.078$ $a(YW)/E_m(YW) = 1.859$ $a(WW) = -0.009$ $E_m(WW) = \pm 0.090$					
N_2 -17- 937	145	55	186	386	6.010 : 2.280 : 7.710
N_2 -17- 953	122	28	140	290	6.731 : 1.545 : 7.724
N_2 -17-1056	113	43	159	315	5.740 : 2.184 : 8.076
N_2 -17-1058	135	42	173	350	6.171 : 1.920 : 7.909
N_2 -17-1200	83	36	131	250	5.312 : 2.304 : 8.384
N_2 -17-1201	109	39	139	287	6.077 : 2.174 : 7.749
N_2 -18- 200	81	22	121	224	5.786 : 1.571 : 8.643
N_2 -17- 939	135	35	177	347	6.225 : 1.614 : 8.161
Total	923	300	1226	2449	6.030 : 1.960 : 8.010
$a(YY) = +0.030$ $E_m(YY) = \pm 0.106$ $a(YW) = -0.040$ $E_m(YW) = \pm 0.072$ $a(WW) = +0.010$ $E_m(WW) = \pm 0.109$					

(4) $F_1(YW) \times WW$

As in the former case, three types of segregation were also found here.

(a) 1YY : 1WW

(b) 1YW : 1WW

(c) 1YY : 1YW : 2WW

These interesting ratios will be discussed in a later section (page 409).

TABLE 15

Lot No.	YY	YW	WW	Totals	Ratios
O_1 -17-1051	31	—	31	62	2.000 : 2.000
O_1 -17-1052	76	—	64	140	2.171 : 1.829
Total	107	—	95	202	2.119 : 1.881
$a = \pm 0.119$ $E_m = \pm 0.095$ $a/E_m = 1.253$					
O_1 -18- 714	—	101	82	183	2.208 : 1.792
O_1 -17-1096	—	41	32	73	2.247 : 1.753
Total	—	142	114	256	2.219 : 1.781
$a = \pm 0.219$ $E_m = \pm 0.084$ $a/E_m = 2.607$					

TABLE 15 (continued)

O ₃ -17-1060	78	63	141	282	1.106 : 0.894 : 2.000
O ₃ -17-1063	80	101	180	361	0.886 : 1.119 : 1.994
O ₃ -17-1094	61	62	119	242	1.008 : 1.025 : 1.967
O ₄ -18-727	74	62	121	257	1.152 : 0.965 : 1.883
Total	293	288	561	1142	1.026 : 1.009 : 1.965

$$a(YY) = +0.026$$

$$a(YW) = +0.009$$

$$a(WW) = -0.035$$

$$E_m(YY) = \pm 0.035$$

$$E_m(YW) = \pm 0.035$$

$$E_m(WW) = \pm 0.040$$

CROSSES BETWEEN YY AND WW

As in the former paragraph, our experiments revealed three different results in spite of the purity of WW used here, remarkable on account of both blood and cocoon being white. The following tables will explain these results.

CASE 1

All F₁'s,—171 cocoons were yielded,—were YY, and they segregated in the ratio of 3YY : 1WW in the next generation.

TABLE 16

Lot No.	F ₂ generation			
	YY	WW	Totals	Ratios
P-17-1189	208	60	268	3.104 : 0.896
P-17-1190	270	67	337	3.205 : 0.795
P-17-1191	231	104	335	2.758 : 1.242
Total	709	231	940	3.017 : 0.983

$$a = \pm 0.017$$

$$E_m = \pm 0.038$$

CASE 2

All F₁'s,—191 cocoons were yielded,—were YY as in the former case, but they segregated in the next generation in the ratio of 9YY : 3YW : 4WW instead of 3YY : 1WW.

TABLE 17

Lot No.	F ₂ generation				
	YY	YW	WW	Totals	Ratios
Q-'18-274	286	55	105	446	10.260 : 1.973 : 3.767
Q-'18-275	182	55	95	332	8.771 : 2.651 : 4.578
Q-'18-277	241	85	123	449	8.588 : 3.029 : 4.383
Q-'18-278	258	94	110	462	8.935 : 3.255 : 3.810
Q-'18-279	181	69	86	336	8.619 : 3.286 : 4.095
Q-'18-280	220	84	116	420	8.381 : 3.200 : 4.419
Total	1368	442	635	2445	8.952 : 2.892 : 4.155

$$a(YY) = -0.048$$

$$E_m(YY) = \pm 0.108$$

$$a(YW) = -0.108$$

$$E_m(YW) = \pm 0.085$$

$$a(YW)/E_m(YW) = 1.271$$

$$a(WW) = +0.155$$

$$E_m(WW) = \pm 0.095$$

$$a(WW)/E_m(WW) = 1.589$$

CASE 3

The 295 F₁ cocoons were all YY as in the former two cases, but two types of segregation in the F₂ generation were found, as shown in table 18.

TABLE 18

Lot No.	F ₂ generation				
	YY	YW	WW	Totals	Ratios
R ₁ -'18-298	326	—	128	454	2.872 : 1.128
Total	326	—	128	454	2.872 : 1.128

$$a = \pm 0.128$$

$$E_m = \pm 0.055$$

$$a/E_m = 2.327$$

R ₂ -'18-295	210	69	115	394	8.528 : 2.802 : 4.670
R ₂ -'18-296	178	74	69	321	8.872 : 3.688 : 3.439
Total	388	143	184	715	8.683 : 3.200 : 4.117

$$a(YY) = -0.317$$

$$E_m(YY) = \pm 0.200$$

$$a(YY)/E_m(YY) = 1.585$$

$$a(YW) = +0.200$$

$$E_m(YW) = \pm 0.158$$

$$a(YW)/E_m(YW) = 1.266$$

$$a(WW) = +0.117$$

$$E_m(WW) = \pm 0.175$$

SILK GLANDS OF YY, YW AND WW

If we cut the back of a mature worm, we find that a pair of silk glands fills up nearly the whole space of the body cavity and looks yellowish or whitish according to their blood color. Though no distinction in the relative intensity of the yellowish color of the blood between YY and YW can be seen with the naked eye, we can easily distinguish the silk gland of YY from that of YW by its deep yellow. The distinction becomes far more obvious if examined in the following manner. Cut the

middle division of the gland with a knife, hold its anterior part firmly between two fingers, suspend it, and pull out the liquid silk carefully from the gland-lumen with the tip of a pincette, then we can separate the transparent silken column from the gland proper.

The silken column of YY thus obtained is deep yellow, while that of YW is white; and further we see that both of their glands proper are equally tinged with light yellow.

From these observations, the two following conclusions can be drawn:

- (a) The color of the liquid silk is quite in accordance with the color of the cocoon fibre irrespective of the blood color; and
- (b) The gland proper is equally tinged with light yellow in the yellow-blooded silkworms no matter whether their cocoon fibre spun be yellow or white.

GENERAL CONSIDERATIONS

In considering the general hereditary phenomena above mentioned, in the first place, the numerical ratios obtained in the experiments may be regarded as trustworthy; for in most of them the deviations from expectation are smaller than the probable errors, and even in the reverse cases the deviations are far smaller than 3 times the probable errors. In the second place, it will be noted that, in disagreement with the studies of TOYAMA and of TANAKA, two factors are necessary to yield the yellow cocoons, as shown by a glance at the yellow-blooded white-cocoon-spinners and their silk glands, i.e., YW.

As shown in case 1 of the crosses between YW and WW, we had YY only in the F_1 generation and $9YY : 3YW : 4WW$ in F_2 . All that is essential to the production of this ratio in F_2 is that F_1 be heterozygous for two factors, of which one is perceptible whenever existent, while the other needs the existence of the first one in order that its own effect may be manifested. Here we represent by C and Y two factors or genes for the yellow-blooded yellow-cocoon-spinners. Whenever C exists, we see the blood colored; while Y alone is not perceptible, but the yellow cocoons are produced by the yellow determiner, Y , only when C is present. Then we have the formula $CCYY$ for YY and $CCyy$ for YW.⁴ In the crosses be-

⁴ Since the completion of this manuscript, a report by Miss L. C. MAUDE on the color characters of the cocoons of the silk-worm appeared in the August number of the Proceedings of the Zoological Society of London, 1918. Miss MAUDE did not determine the color character of the cocoons during the larval stages, but examined the cocoon colors only, and tried to represent the character of the yellow by a factor Y , and that of the flesh by a factor F .

As regards the various colors of the cocoons with the exception of the yellow dealt

tween YW and YY we have therefore $CCYy$ in F_1 and consequently in F_2 we should have the segregation of the simple three-to-one sort. This expectation has been fully realized. There will be no need of illustrating the segregating phenomena in F_2 and back-crossing of F_1 with both of the pure parent breeds, which are shown in tables 1 to 3.

Three formulae may be conceived for WW, namely, $ccYY$, $ccyy$ and $cCYy$, but possessors of these three formulae apparently can not be distinguished one from another; consequently, in the crosses between YW and WW, and between YY and WW, we ought to have three cases which may be put briefly in tabular form as follows:

The Arabic numerals in parenthesis denote the number of individuals having the indicated genotypic constitution.

If we look at the genetic constitutions of YY, YW and WW of F_2 generation enumerated above, it will be easily seen that our expectations in F_2 should be as follows:

1. An F_2 generation produced by YY should be of four kinds, namely, those breeding true to YY ($CCYY \times CCYY$; $CCYY \times CCYy$; $CCYY \times CcYY$; $CCYY \times CcYy$; $CcYY \times CCYy$);⁵ those segregating in the ratio of 3YY:1YW ($CCYy \times CCYy$; $CCYy \times CcYy$); those segregating in the ratio of 3YY:1WW ($CcYY \times CcYY$; $CcYY \times CcYy$); and those segregating in the ratio of 9YY:3YW:4WW ($CcYy \times CcYy$).

2. An F_2 generation produced by YW should be of two kinds, namely those breeding true to YW ($CCyy \times CCyy$; $CCyy \times Ccyy$), and those segregating in the ratio of 3YW:1WW ($Ccyy \times Ccyy$).

3. An F_2 generation produced by WW should all breed true to WW, though their genetic constitutions might differ.

These expectations were all fulfilled as is shown in table 5.

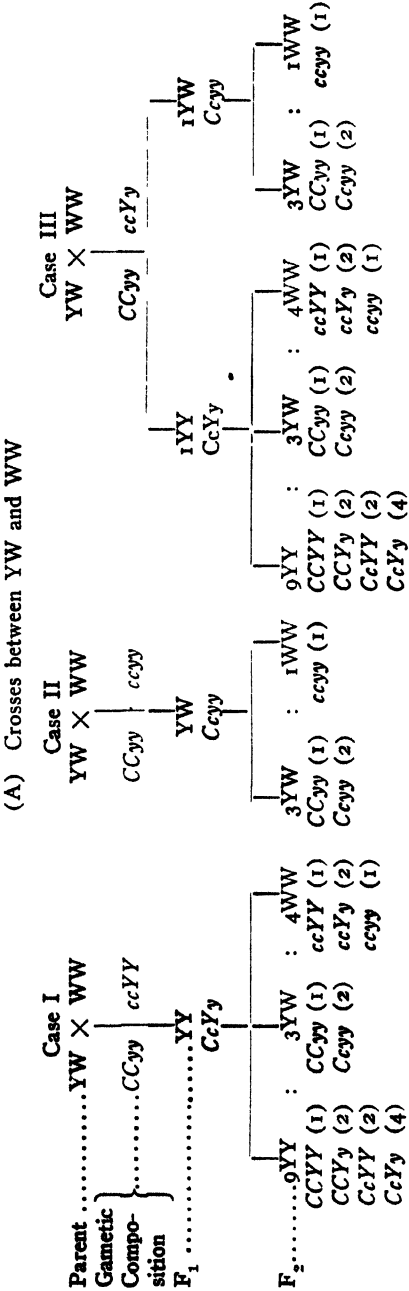
The results from the back-crossing of F_1 with both of the pure parent breeds still further support our conclusions, but there is no need of detailed explanation, for tables 6 and 7 give sufficient proof.

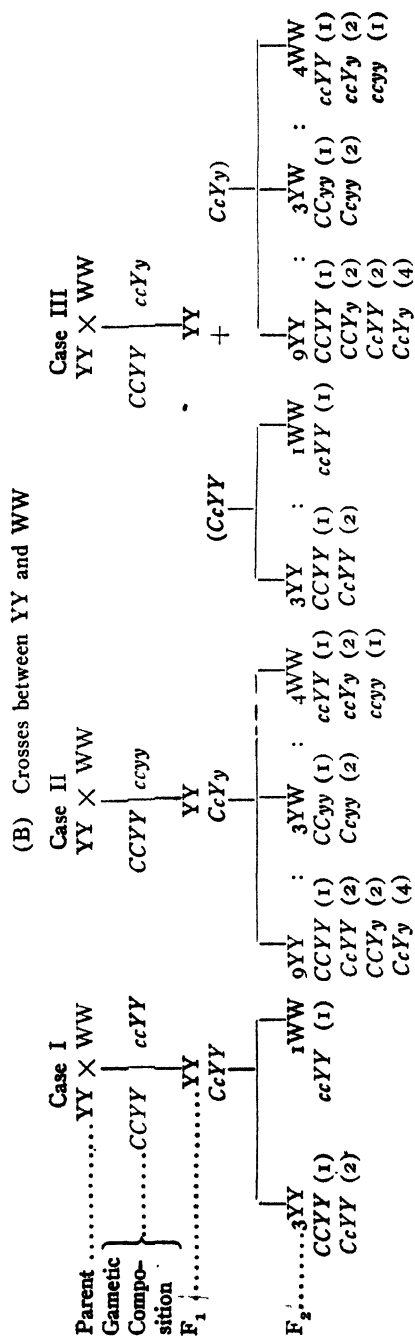
These tabular expectations were also completely fulfilled as shown in tables 15 to 18.

It is thus clear that all of the new observations are explained if two with in the present paper, many experiments have been already carried out, which although not yet completed, make it possible to state with certainty, that the flesh color of the cocoons is produced not only by the factor *F* as Miss MAUDE is inclined to think, but by the presence of another factor which we call *C*.

Descriptions of the results obtained by these experiments as well as discussions upon them will be treated in a separate paper.

⁵ These comprise all the possible crossings which may yield the mentioned F_2 .





factors or genes are assumed to be necessary for the production of the yellow cocoon, one of these factors being perceptible whenever present, giving the blood a yellow color, while the other needs the presence of the first in order that its own effect may be seen by making the cocoon yellow also. In the words of SHULL (1908) the relation of these two factors represents a case of "latency due to separation," since patency is brought about by recombination of C and Y . The F_2 ratio 9:3:4, consequent upon the above presumption, recurs very frequently in Mendelian analyses.

In general, for instance, we assume two genes X and Y , and that X is a gene which, independently of other known factors, produces a perceptible effect, and that Y needs the presence of X in order that its own effect may be manifested. Although there is no apparent distinction in the pure-bred xx —, they differ genetically with respect to the gene Y , assuming the three types $xxYY$, $xxyy$ and $xxYy$. But very few investigators have shown this relation systematically, though there are many elaborate studies on the "compound characters," the "plural genes" or "several types of latency." So I shall discuss it somewhat more particularly.

In the spring of 1917, I made reciprocal crosses between a Chinese di-voltine white race called "Shōzan" and the F_1 hybrid of YW and YY already mentioned. The former had been bred true to WW since 1914, and the latter is doubtless $CCYy$ in its genetic constitution if our theory is right. From this crossing the following results were obtained.

TABLE 19

Lot No.	YY	YW	Totals	YY : YW per 4	a	E_m
S-'17-961	367	—	367	All YY	—	—
S-'17-969	186	190	376	1.979 : 2.021	± 0.021	± 0.070
S-'17-962	317	—	317	All YY	—	—
S-'17-971	303	106	409	2.963 : 1.037	± 0.037	± 0.058

These various results will be clearly understood if three kinds of genetical constitution are assumed in the "Shōzan," namely, the "Shōzan" used in No. 961 and No. 962 is $ccYY$ and that of No. 969 is $ccyy$ and that of No. 971 is $ccYy$. These results show that there are three kinds of WW even in a race which is supposed to be one.

The results above mentioned may, on other points of view, be explained by the relation of activity between one character and its antagon-

istic character according to DE VRIES, or by the same genesis which causes the modification of genetic factors, which incidentally results in a change from homozygosis to heterozygosis as EMERSON illustrated in variegated ears of maize.

But we consider that these discussions treat rather of the origin of the gene Y ; so we shall only lightly touch them. Here I will confine myself to the statement that the three distinctions in WW were in existence, though not discovered up to the present; further, that we can find no modification between $ccYY$ and $ccyy$, and that there is no change from each of them to $ccYy$ which was ascertained by crossing them with $CCyy$ for six generations in succession.

SUMMARY

1. A new race in *Bombyx mori* which spins white cocoons notwithstanding the fact that its blood is yellow, was found by crossing *inter se*, to breed true to its type.
2. The color of the liquid silk is quite in accordance with the color of the cocoon fibre, irrespective of blood color, and the gland proper is equally tinged with light yellow in the yellow-blooded silkworms, no matter whether their spun cocoon fibre be yellow or white. This disagreement of the blood color of the silkworms and that of cocoons requires necessarily and sufficiently two factors or genes, C and Y , to yield the yellow cocoons.
3. The gene C is perceptible, whenever present, by making the blood yellow, while the other, Y , alone, is not perceptible. But it is necessary for the yellow determiner Y to exist with C to yield the yellow cocoons. This supposition has been fully sustained by the examination of the F_1 , F_2 , F_3 and back crosses of the F_1 hybrid with both of the pure parent breeds, in crosses between YW and YY , between YW and WW , and between YY and WW .
4. Although there is no apparent distinction in pure bred WW , they differ genetically with respect to the gene Y , assuming the three types $ccYY$, $ccyy$ and $ccYy$. This fact has been fully demonstrated in a Chinese di-voltine white race called "Shōzan" which had been bred true to WW since 1914.
5. Two kinds of WW , namely, $ccYY$ and $ccyy$, were found by crossing *inter se* to breed true to their types. No modification was found between $ccYY$ and $ccyy$ and no change from either of them to $ccYy$, which was ascertained by crossing them with $CCyy$ for six generations in succession.

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A STUDY OF STERILITY IN THE PLUM¹

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INTRODUCTION

In a former publication on weather in relation to fruitfulness in the plum (DORSEY 1919) it was shown that during bloom weather conditions may be such as alone to prevent the setting of fruit. It was further emphasized that, aside from the total effect of weather, certain single factors of it, acting in the extreme, might be singled out as being responsible for the failure of fruit to set. Weather conditions were shown to have their most immediate influence on such processes as dehiscence, pollination and fertilization. These conclusions are further supported by the variation in the extent of the second drop from year to year which can in general be correlated with weather conditions at bloom.

Aside from the influences affecting the functioning of the organs of reproduction which can be assigned to interference from weather or from the environment, there are others having a direct bearing upon reproduction, and hence upon fruitfulness, which appear to act within the germ plasm and are therefore inherent. An investigation of these influences forms the basis of this report.

THE STATUS OF SELF-STERILITY IN THE PLUM

Former investigators have dealt primarily with the economic phase of sterility. Tests have been made in *Prunus americana*, *P. Besseyi*, *P. hortulana*, *P. nigra*, and *P. triflora*, by WAUGH (1896, 1897, 1898, 1899), GOFF (1894 and 1901), HEIDEMAN (1895), WAITE (1905), and others. These show that the cultivated varieties of native species, with two exceptions, New Ulm (HEIDEMAN 1894) and Robinson (WAUGH 1898), are self-sterile. A similar condition was found in some of the Sweet Cherries by GARDNER (1913). On the other hand, the work of BACKHOUSE (1911 a, b), PETERS (1916), and SUTTON (1918), shows that in *P. domestica* only about one-half of the varieties are self-sterile.

It should be stated here that self-pollination and self-sterility are used with reference to the clone. Self-pollination in botanical usage refers to the transfer of pollen from an anther to the pistil of the same flower. In horticultural usage self-pollination has a broader sense and includes the transfer of pollen from any flower borne by a variety to any pistil of the same variety. Likewise self-sterility refers to the clone rather than to the flower or individual plant.

Since self-sterility has been found to be so prevalent, considerable interest centers around the reliability of the tests which have been made. WAUGH (1898) discusses this phase of the subject in some detail and concludes that the method used,—that of covering the blossoms with paper bags or other material,—is reliable. His conclusions agree with those of WAITE (1894) and BEACH (1898, 1899). This point was considered of sufficient importance to be checked further by different methods on account of its commercial as well as its scientific bearing. The data obtained in this test are presented in table I. Bags were not used either on the trees grown in tubs in the greenhouse or on those tented in the orchard, so that any adverse influence the bags may have had in previous tests was eliminated in these. ALDERMAN (1917) in sterility studies in the apple, followed a similar method by covering the entire tree with muslin.

It will be noted that *P. americana*, *P. americana mollis*, *P. Besseyi*, *P. domestica*, *P. hortulana*, *P. nigra*, and *P. triflora* are represented in this table. The results with the trees under the tent as well as with those in the greenhouse agree with those previously reported and show that self-sterility is the outstanding feature of all the varieties included in these tests and that it is constant in expression. Considering the numbers under observation, the few exceptions found may be regarded as within the limits of experimental error, and it is even possible that some may have been self-fertilized, since, as will be shown later, pollen-tube growth takes place under these conditions. It appears safe to conclude therefore that the general condition in this genus has been correctly reported.

Since self-sterility is so general in the plum, cross-pollination, except as noted in certain varieties of *P. domestica*, is essential to fruitfulness. This makes it necessary to give careful attention to the blossoming-dates of varieties used as pollenizers. Such a classification of varieties has been compiled by a number of workers: WAUGH (1896, 1898, 1900); GOFF (1901); HEDRICK (1908), and others. In addition to planting pollenizers which bloom at the same time as the variety to be pollinated,

TABLE I

Showing the degree of self-sterility in selected varieties and species tested in the greenhouse and tented in the orchard as a check to the method previously used.

Variety	Condition of growth of trees, in self-pollination tests	No. of flowers pollinated	No. of fruits set	No. of fruits mature
Burbank	In greenhouse	Entire tree	6	0
"	"	1 branch	0	0
Compass	"	Entire tree	0	0
Minnesota No. 6 ¹	"	185	1	0
" " "	"	24	0	0
" " "	"	1 branch	—	1
Minnesota No. 10 ¹	"	1	0	0
" " 12 ¹	"	49	0	0
" " 21 ¹	"	1 branch	0	0
" " 35 ²	"	84	2	0
<i>P. Besseyi</i>	"	2 trees	0	0
Surprise	"	Entire tree	0	0
Yellow Egg	"		10	4
Minnesota No. 8 ²	Tented in orchard, 1915	Entire tree	0	0
" " 21	" " " "	" " "	0	0
Sand Cherry × Apricot	" " " "	" " "	0	0
Wolf ³	" " " "	" " "	0	0
Minnesota No. 9 ¹	" " " 1916	" " "	0	0
" " 12	" " " "	" " "	0	0
" " 21	" " " "	" " "	0	0
Assiniboin	" " " 1917	" " "	0	0
Minnesota No. 8	" " " "	" " "	0	0
" " 21	" " " "	5-yr.-old tree	0	0
Compass	" " " "	Entire tree	0	0
Etopa	" " " "	1 branch	0	0
<i>P. americana</i>	" " " "	4-yr.-old tree	0	0
<i>P. Besseyi</i> , Tree No. 1 ⁴	" " " "	281	1	0
" " " 2 ⁴	" " " "	263	2	—
" " " 3 ⁴	" " " "	176	8	—
" " " 5 ⁴	" " " "	300	1	—
Wakapa	" " " "	6-yr.-old tree	0	0
Wohonka	" " " "	6-yr.-old tree	0	0

¹ Cross between Burbank and Wolf.

² Cross between Abundance and Wolf.

³ Large percentage of pistils aborted.

⁴ The tents covering these trees blew off during a heavy rain on May 21st.

the effectiveness of the variety selected as a pollenizer, or the mutual "affinity" with the variety to be pollinated, must be determined. WAUGH (1899), HEIDEMAN (1895) and others have given this point some study. It is sufficient to state here that while there are differences in the effectiveness of pollenizers, inter-sterility has not been found to be extensive

in the varieties of the native species, although *P. domestica* cannot be pollinated successfully with the native varieties. From the economic standpoint, therefore, the essential facts in the control of sterility in orchard plantings as a means of avoiding crop failure are already well understood.

It may be stated at this point that unfruitfulness is not considered herein from the standpoint of injury due to fungous diseases and insects.

Since the prospect of a crop, so far as the setting of fruit is concerned, may be determined by inspection as early as the five- or six-week period after the time of bloom, it will be seen that the problem of sterility, while limited in point of time, covers that period in the life cycle when delicate sex structures must not only form but must function. Consequently greatest emphasis has been given to this period in order to determine what factors are operating in this genus, not only to bring about self-sterility so extensively, but also to reduce to such an extent the number of functional pistils found in some seasons. The successive main headings will indicate clearly the phases of the problem covered.

MATERIAL AND METHODS

A representative list of varieties and hybrids has been available for this study. A part are growing in the experimental orchards at University Farm and the remainder at the Fruit-Breeding Farm six miles west of Excelsior, Minnesota. In both orchards the trees are grown under clean cultivation. Attention has been given to the species as well as to the variety. HEDRICK *et al.* (1910) have been taken as authority for the species of the different varieties except in a few of recent origin.

In the cytological investigations the usual technique has been followed. As to the killing fluids, chromo-acetic and Flemming's medium were most used, Carnoy's fluid being a poor fixative for the plum. The triple stain and Heidenhain's iron-alum-haematoxylin both proved to be excellent stains. Although the cytological phase of sterility is presented briefly, material was fixed and sectioned extensively. In all, over 2600 preparations were made in covering the different stages in anther and pistil.

POLLEN DEVELOPMENT IN RELATION TO STERILITY

Since functional pollen bears such a vital relation to fruitfulness, a careful cytological study of pollen development in the plum has been made with the object of determining the general condition in this genus.

With a knowledge of the pollen condition in species, hybrids, and varieties at hand, the relation of pollen to self-sterility can be ascertained. Moreover, normal development will serve as a basis for comparison in determining type and extent of pollen abortion.

Normal pollen development

Pollen development was studied in detail in varieties representing *P. americana*, *P. nigra*, *P. triflora*, *P. domestica*, *P. pennsylvanica*, *P. hortulana* Mineri, and *P. Besseyi*, but since there was so little variation from the condition common to the higher plants only a very brief description of the earlier stages will be included here. The later stages, however, especially those beyond the point of pollen degeneration, will receive more detailed treatment.

The winter stages

In Virginia, DRINKARD (1910) found that during December and January there was a slight development going on in the fruit bud of the plum. On January 10th in Abundance the pollen mother-cells were in the resting stage. As early as February 12th, there was some indication of division and by February 24th tetrads were being formed.

In Minnesota pollen development is less advanced during the winter. In Surprise on December 11th, development had progressed no farther than the archesporial-cell stage, and on March 23rd no further growth had taken place. By April 12th, the synaptic stage had been reached. Burbank and *P. nigra* on March 22nd were at the early archesporial-cell stage and it was not until April 6th that Burbank had formed pollen mother-cells which one week later were at synapsis. Material was fixed March 22nd, 1915, from varieties representing *P. triflora*, *P. americana mollis*, *P. hortulana* Mineri, and a number of hybrids between *P. triflora* and *P. americana mollis*, and in none had development advanced farther than the archesporial-cell stage. On the other hand, on January 13, 1918, *Amygdalus Davidiana* had pollen grains with two nuclei, but with scant cytoplasm.

The anther wall

During the early archesporial-cell stage of the winter months the central cells of the anther are somewhat larger than those of the outer three rows and differ from them primarily in having larger nuclei and more angular walls. The cells of the epidermal layer are small and have a staining reaction similar to the others.

At the pollen mother-cell stage the anther walls are three to four cells

thick and noticeable elongation has taken place in the outer layer, while the three inner layers, particularly the innermost, are somewhat compressed. The cells of the outer layer take the orange, while the other layers, like the tapetum, have a greater affinity for the violet. The partition between the loculi of an anther is also three to four cells thick, and these cells as early as the open spireme stage are very much compressed and elongated.

In the final growth stages, marked changes take place in the cells of the anther wall. The outer wall of the epidermal cells becomes thicker, and additional elongation takes place as the anther cavity increases in size. The cells of the hypodermal layer become much broader, but show the most marked change from the earlier stages in their greater length and in the presence of conspicuous ridges in the wall. This layer is the most prominent element of the mature anther wall and is bordered on the inner side by the collapsed and very much extended walls of the inner layers. The cell layers between the loculi, which have become very much compressed at the liberation of the microspore, disappear with the tapetum,—a change which throws the pollen of both loculi together. The point of union of the partition between the loculi with the outer wall marks the place of dehiscence. In fact, at the time of the dissolution of the partition cells a part of the cells of the anther wall along the line of the suture is also dissolved.

The early pollen stages

The tissues of the anther at the pollen mother-cell stage have the characteristic differentiation and staining reaction. In cross section, the mother-cells are four to five cells deep and four or five times as long. The polyhedral walls about them are thin at first but become noticeably thicker previous to rounding up. The uninucleate tapetal cells are slightly larger than the mother-cells and stain more deeply with the blue.

The chromatin in the winter stages of the archesporial cells is coarsely granular and the relative uniformity of the deeply staining masses in number and size is striking. The number of these bodies approximates that of the double number of chromosomes.

The rather scant chromatin of the mother cells, however, in the early stages is finely granular and quite evenly distributed throughout the nucleus. Previous to the formation of the spireme thread, larger and more deeply staining masses are found. The spireme which enters synapsis is very slender, irregular in marginal outline but distinctly

granular. The synaptic mass is very tight and compact, being in many cases but little larger than the nucleolus near or about which it is typically located.

At the time the synaptic mass is unraveling, a few short loops appear first. These gradually lengthen and with further loosening others are formed so that the thread is soon spread throughout the nuclear area. While the plum is not suitable material in which to study the manner of pairing of the chromosomes, well fixed preparations of the spireme at critical formative stages show a condition which strongly favors the side-by-side pairing.

During the open-spireme stage the chromatin thread increases in thickness and as diakinesis is approached becomes much looser and even more granular. Immediately following segmentation the chromosomes are very irregular in outline and in many cases the individuals of a pair lie distinctly apart. With further development, they gradually become more compact and at the end of this stage are found evenly distributed near the nuclear membrane. As the time of division is approached the chromosomes assume a homogeneous structure which completely obscures their double nature.

The heterotypic and homoeotypic division

Coincident with the appearance of the spindle fibers of the heterotypic division, the nuclear membrane becomes irregular in outline and the area of the nucleus much smaller. The multipolar spindle of the early preparatory changes forms a distinctly bipolar spindle at the metaphase. The chromosomes at the equatorial plate lie in slightly different planes in most of the preparations of this stage, and show some irregularity in the passage to the poles. The fibers of the spindle, particularly the intra-polar fibers, are very distinct. Following this division the chromosomes are drawn together at the poles in a close, compact mass. The heterotypic spindle gradually becomes less distinct as the nuclei of the dyad are formed.

After reorganization, the dyad nuclei divide simultaneously. The spindle of this division is slightly smaller and narrower at the equatorial-plate stage than is that of the first division, and the chromosomes are smaller although distinct. The spindles sometimes lie in the same plane although typically they are in planes perpendicular to each other. The chromosome number determined in the plum is presented in table 2. Judging from the number of species represented, ten chromosomes as the reduced number is quite common in this genus.

TABLE 2
Showing the number of chromosomes found in different forms
of the plum.

Variety	Chromosome number
Iron Clad (<i>P. americana</i>).....	10
Minnesota No. 12 (<i>P. triflora</i> × <i>P. americana mollis</i>)	10
Opata (<i>P. Besseyi</i> × (<i>P. Munsoniana</i> × <i>P. triflora</i>))	Near 10
<i>P. pennsylvanica</i>	20 2x
Stella (<i>P. americana</i> × <i>P. triflora</i>)... ..	10
Stoddard (<i>P. americana</i>)	10
Surprise (<i>P. hortulana Mineri</i>).....	20 2x
Wolf (<i>P. americana mollis</i>).....	20 2x
Wyant (<i>P. americana</i>).....	0

From the above description it will be seen that pollen development proceeds through the heterotypic and homoeotypic divisions with every appearance of being normal. This condition obtains for the most part in the varieties of pure species as well as in extreme hybrid forms. Degenerative processes which become so active later do not gain expression as early as this.

The tapetum

The tapetum in the plum shows no marked variation from its usual course of disintegration. Its cells have a single large nucleus which first divides about the time of the first division in the mother-cell. The most noticeable changes which take place in the tapetum previous to the liberation of the microspores are vacuolization and further division of its nuclei. In some anthers advanced degeneration takes place at the tetrad stage, at a time when the walls are still intact. Following the liberation of the microspores, the tapetum rapidly disappears. During the period of rapid anther enlargement, while its cells are yet intact, the tapetum is often withdrawn from the anther wall. Following this stage the tapetal cells are more or less separated and undergo the most rapid disintegration. The walls about the tapetum persist much later than the mother-cell wall which disappears typically at the late tetrad stage. In the mature anther only occasional traces of tapetal cells or walls remain in the anther sap. The functioning of the tapetum and its disappearance from the anthers in which there is partial or complete pollen abortion are the same as in those bearing all normal grains. The tapetum in the plum, then, functions normally as nourishing cells and apparently has no bearing upon pollen degeneration.

The tetrad wall

Tetrad formation marks the point at which the hereditary allotment to each nucleus has been made and also the beginning of an independent existence of each microspore. The reaction of the microspore to its environment, both before and after liberation, therefore, is of particular interest from the standpoint of aborted pollen.

The stages in the formation of the wall about the rounded mother-cell in the plum are very distinct and can be easily followed. The origin of this wall in other forms has been given some attention but its relation to the wall of the mother-cell has not always been indicated. According to the evidence at hand there appears to be two distinct methods of disposing of the mother-cell wall: (a) in one case, as in the lily (ALLEN 1905) and the grape (DORSEY 1914), the original mother-cell wall disappears at the rounding-up stage; and (b) in the other, illustrated by the strawberry (VALLEAU 1918), the mother-cell wall remains intact after rounding-up has taken place. The plum belongs to the latter class and since these early stages precede the action of degenerative processes they will be presented in some detail.

The angular walls between the early mother-cells have the appearance in section of thin lines. These take the orange heavily with the triple stain and with Heidenhain's haematoxylin stain light or dark blue. At this time they are similar in thickness to the walls of the tapetum. At the time of the rounding up of the mother-cell the walls become noticeably thicker than those between the tapetal cells, but the staining reaction is similar.

The first evidence in the plum of a new wall about the rounding-up cytoplasm of the mother-cell is the separation of a thin layer from the inner surface of the old wall, first at the cell angles and subsequently farther along the sides (plate 2, '1). In some sections the new wall,—which will hereafter be referred to as the *tetrad wall* as distinguished from the persistent mother-cell wall,—appears as a line and in others as a surface, according to the angle of view. Later stages show considerable irregularity in separation. At the cell angles and narrow ends of the cell, it is generally drawn away while yet in contact with the old wall along the longer sides. From the irregularity in separation, however, it should not be inferred that there is necessarily a similar irregularity in formation, since, where partly separated or even in contact with the old wall, its outline can generally be followed distinctly around the remainder of the cell periphery. Complete separation of the tetrad wall takes place in most cases previous to the heterotypic division.

The tetrad wall appears to be a derivative of the mother-cell wall instead of the cytoplasm. This view is supported by the manner of separation as well as by the evidence from staining reaction. As noted above, in the young mother-cell the walls have the appearance in section of a thin line and later undergo slight thickening and stain heavily with the orange. Immediately after the separation of the tetrad wall, which stains a light orange, the mother-cell wall, which is again noticeably thinner than just previously, has a darker reaction to the orange. Furthermore, since the cytoplasm rounds up first, the plasma membrane is very distinct and separated in places from the tetrad wall which, at this time, is generally completely formed.

The mother-cell wall, which remains after the formation of the tetrad wall, persists in many anthers as late as the liberation of the microspores. In others it disappears soon after the heterotypic division. The rapid increase in the size of the anther cavity brings about considerable elongation in the mother-cell wall which provides ample space for the tetrad. The tapetal cell walls disappear later than those of the mother-cell so it appears that different enzymes are acting, or if a single enzyme, that dissolution is localized.

The tetrad wall, which is thin and homogeneous when first separated, does not undergo any appreciable thickening until after the heterotypic division. Subsequent to this, particularly following the organization of the tetrad nuclei but coincident with wall formation between them, the tetrad wall increases rapidly in thickness. The maximum thickness of the outer wall is found when the walls between the microspores reach the greatest thickness, i.e., in the mature tetrad.

Traces of cell plates appear between the tetrad nuclei by the deposition of material near the central point of the intra-polar spindle fibres. The staining reaction of this material is at first slightly darker than that of the thick tetrad wall and the tetrad nuclei also round up before the walls between them become thick. Further rounding of the spores is followed by a gradual entrance of the viscid tetrad-wall between them until each one is completely enveloped with the thick wall characteristic of this stage (plate 2, 2). The stages in the division of the cytoplasm between the microspore nuclei of the tetrad have been followed out in considerable detail and for the most part agree with the observations of FARR (1916, 1918). The question now enters as to what differences appear in the morphology of the tetrad as a result of the two types of mother-cell wall dissolution, i.e., a dissolution of the middle lamella at the time of tetrad-wall formation as in the grape, and dissolution subse-

quent to the heterotypic division, as in the plum and strawberry.

In the grape, plum and strawberry, the outer wall between a mother-cell and a tapetal cell persists after rounding-up has taken place in the cytoplasm. In this position the tetrad wall has a similar origin in each and a careful study of these stages shows a similar structure and separation. In all three forms this wall, which is very thin at first and subsequently thickens, the interpretation appears justified that it is homogeneous throughout because the stains used did not show in either that the exterior is bordered by a thin wall or membrane. Such an interpretation is in accord with the well known swelling of colloidal substances. The refractive power of the outer margin of the tetrad wall in the grape and plum is identical with the triple stain and is similar to that of the inner layer about the microspores. Furthermore, there is never a separation of an outer wall or membrane from an inner thicker portion of the tetrad wall and during dissolution there is no appreciable persistence of either the outer or the inner surface over that of the middle portion. It is possible, however, that other staining methods may show differentiation in the tetrad wall not revealed so far by the technique used. It appears then that the differences in the time of dissolution of the mother-cell wall result in no striking morphological differences in the tetrad wall. In the grape, that remnant of the mother-cell wall, whether simply the middle lamella or more, which does not enter into the formation of the tetrad wall is dissolved immediately, while in the plum and strawberry it persists for a time longer. In one case there appears to be an enzyme action which is either absent or delayed in the other. The origin of the tetrad wall, however, appears to be identical in each type.

The microspore wall

The microspore wall first appears in section as a very fine line between the plasma membrane and the thick tetrad wall (plate 2, 2). It is best seen where slight plasmolysis has occurred. Before the tetrad wall is dissolved the microspore wall becomes noticeably thicker. The sequence of events is such that the extended cross walls of the mother-cell, the tetrad walls, and the walls about the microspores can sometimes be seen in the same anther. The microspore wall is formed adjacent to the interior surface of the thick tetrad wall but outside of the plasma membrane. No evidence of it can be seen in the plum until after the tetrad wall has become thick between the microspores, but since the inner border of the tetrad wall about each nucleus at this stage stains slightly more heavily than previously the interpretation is that at least a part of the

heavier staining reaction is due to the changes taking place in microspore-wall formation.

The liberated microspore

Before the microspore is set free in the anther sap its nucleus is re-organized and in the resting stage. In many anthers the microspores of a tetrad remain in their usual position in relation to each other for some time after the dissolution of the tetrad wall. Breaking down of the persistent mother-cell wall generally precedes the dissolution of the tetrad wall although both may disappear at the same time. In the plum as in *Fragaria* (VALLEAU 1918) there is no appreciable increase in size in the microspores before liberation. The anther sap is clear and homogeneous at the time of dissolution of the tetrad wall, and its staining reaction is not changed by the inclusion of the substance of the walls of the mother-cell and of the tetrad.

The germ pore is formed in the plum microspore immediately after liberation from the tetrad, during the early stages of thickening and growth in the microspore wall, but before the time of rapid extension. The first evidence of the suture is a bending in of the previously spherical covering forming three longitudinal grooves in the surface at places where the wall appears slightly thinner. During this stage the two distinct elements of the wall can be for the first time definitely distinguished. At the germ pore the intine is continuous and the exine in cross-section is broken or discontinuous. Further thickening takes place primarily in the exine, which in the mature pollen grain constitutes the most conspicuous part of the wall. There are three sutures and at the mid-point of each a germ pore. The germ pore is bordered by projecting, fimbriated outgrowths of the exine (plate 2, 3) which are considerably raised and are conspicuous in the mature pollen. These edges overlap, and in some varieties, as Wyant, the pore is closed by them. The germ pore is present in all forms included in this investigation, a condition which is very different from that in the grape in which it is absent in all pollen borne by reflexed stamens.

Subsequent to the formation of the germ pore the microspore wall enters a period of rapid growth both in extension and thickness. This takes place much more rapidly than the growth of stainable cytoplasm, producing that appearance characteristic of this stage in which large vacuoles are formed. The stainable cytoplasm, with the nucleus, is located mostly toward one side.

The general relation of wall, vacuoles and cytoplasm is maintained until the division of the microspore nucleus, a condition which results in the division figure being located at one side in the rather narrow crescent-shaped cytoplasmic mass. So far as observed, the division figure is perpendicular to the microspore wall, and the cell plate cuts off the typical small generative cell. Division is followed by a further increase in the size of the pollen grain in which there is a rapid growth of the stainable cytoplasm.

The mature pollen grain

The exine of the mature pollen grain is thick and its exterior is marked by prominent ridges and furrows (plate 2, 4). These are most conspicuous in *Prunus americana* and *P. nigra*; in other species, as *P. virginiana*, *P. pennsylvanica* and *P. Besseyi*, the surface is only slightly furrowed although distinctly rough. The sutures are prominent and extend nearly the entire length of the grain. The protrusions about the pore also vary in development in the different species, some extending only slightly over the pore and some practically covering it. In some sections the two coats in the wall can be clearly distinguished by a difference in staining, although they are very seldom separated except where cutting has been the cause.

Soon after nuclear division in the microspore there is a rapid increase in the stainable cytoplasmic content so that at maturity the conspicuous vacuoles of the earlier stages disappear. At the time that the stainable cytoplasm completely fills the space within the wall of the pollen grain the microspore nuclei reach their maximum size (plate 2, 17).

In the mature pollen grain the nuclei, particularly the generative nucleus, are characterized by their small size (plate 2, 8). This diminution in size is brought about by the withdrawal of nuclear sap, a process which first becomes evident by the irregular outline of the nuclear membrane. The staining of the nuclei when thus contracted is clear and distinct and not diffuse. There appears to be a concentration of the chromatin into larger masses as contraction progresses from the more finely granulated condition found immediately after the telophases of the division (plate 2, 9). The lightly staining network, which connects the finely granular chromatin of the earlier stages, becomes less conspicuous at maturity. This is the normal condition in forms known to produce viable pollen.

The generative cell as well as the generative nucleus decreases markedly in size as maturity is reached, as will be seen by comparing figures

5, 6, 7, 8, 9, and 10, of plate 2. In some sections the limiting membrane of the generative cell is so closely contracted about the nuclear membrane that the generative cell has the appearance of a nucleus (plate 2, 9 and 10). It may be either round or lens-shaped, and while generally located near the center of the pollen grain close to the vegetative nucleus it is in some cases found at one side adjacent to the wall. Conspicuous features of the contracted generative nucleus at pollen maturity are the dense masses of chromatin, relatively few in number, and the extremely small nucleolus (plate 2, 8). The position of the generative cell in the cytoplasm is independent of that of the germ pores. In Yellow Egg (*P. domestica*) the generative cell is larger than in the American species, and at maturity the chromatin is more finely granular. In Wyant and Iron Clad, the generative cell is usually small when first cut off.

Pollen is mature before the stigma, and owing to its maturity and the protection afforded by its thick wall and the anther wall it is more resistant to adverse weather than is the stigma. At about the time the petals are bursting the nuclei in plum pollen are entering upon the contracted stage, and because of the presence of the anther sap they remain turgid until the drying which accompanies dehiscence. When dry, instead of being spherical and turgid, they are oval in outline and have three deep folds lengthwise in the covering corresponding to the sutures. In some varieties pollen is readily removed by the wind when dry after dehiscence, and in others but little is blown away because of the adhesive action of a yellowish oily substance.

The pollen tube

Upon reaching a receptive stigma, both aborted and normal pollen grains soon become turgid and spherical. When the growth of the tube starts there is a slight bulging of the intine at the germ pore adjacent to the stigma, but the tube nucleus and generative cell still remain in their usual position. When the tube first emerges from the pore, the cytoplasm contained in it and that adjacent in the pollen grain stains more deeply than before germination. In some grains previous to germination the cytoplasm stains more heavily about the margin, near the plasma membrane. The chromatin in both nuclei at this time is finely granular and more homogeneous than before in its staining reaction. The tube is large and conspicuous when first formed and becomes noticeably more slender as it advances into the stylar tissue, and both the tube nucleus and the generative cell, as well as the larger portion of the cytoplasm,

enter it by the time its length is three to four times the diameter of the pollen grain. In the micropyle, after having passed through the tissue of the style, the tube again becomes thicker. MOORE (1917) notes this difference in diameter and attributes it to food supply. It appears, however, to be due more largely to the stage of growth. Since the movement of the generative cell in the tube can be easily followed, division must take place there, although in the numerous sections of the tube at this stage no division figures have been found. In fact in sections of tubes in the micropyle (plate 2, 11) the generative cell can be found still undivided so that it is probable that ♂ gametes are formed late in the period of tube growth.

After the tube has extended as far as one-half of the length of the style its cytoplasm becomes vacuolated and the nuclei are very inconspicuous. Partitions are formed in the tube, although they are not easily found because they do not stain with Heidenhain's haematoxylin or with Flemming's triple stain. Partitions were also found by OSTERWALDER (1910) in the pollen tube of the pear and by KNIGHT (1917) in the apple, but stains were used by the latter which makes a study of this feature much easier than the stains used in this investigation. As the tube advances in the style the cytoplasm is located well toward the growing tip and the empty walls of the tube left behind can be found in the stylar tissue, in many instances still leading to the empty pollen coverings.

Aborted pollen never develops tubes. Empty coverings of grains which have developed tubes in sections of the stigma are readily distinguished from aborted grains by their slightly larger size, the broken intine or remnant of the tube, and the absence of cytoplasm. In all of the preparations of receptive stigmas many normal-appearing grains do not germinate and from many others only short tubes are formed (plate 5, L and M); yet these are under conditions where others grow normally. This condition prevails in controlled crosses as well as in cases of controlled self-pollination where all pollen is known to have been applied at the same time. In the style the tubes become fewer in number toward the base while immediately beneath the stigmatic surface there are in many cases a multitude of short tubes, which in length grade gradually into the longer ones. The great extremes in the rate of growth shown by the tubes from different grains in the same style account for the small number found at the micropyle in the later stages. Later more attention will be given to the significance of the series presented here.

From the foregoing it will be seen that normal pollen development is the typical condition in the plum. Self-sterility and cross-sterility, which are so general, are not due to degenerate pollen except in those forms where pollen abortion is complete. From plate 5, L, it will be seen that there is tube growth when the plum is self-pollinated, so it may be definitely stated that self-sterility is caused by other factors which operate subsequent to tube formation. At this point interest centers around the extent to which aborted pollen modifies the typical pollen condition in the plum.

Aborted pollen or arrested development

The types of aborted pollen

In *Prunus*, as in *Vitis* (DORSEY 1914) and *Fragaria* (VALLEAU 1918), the range in the time of pollen abortion extends from liberation from the tetrad to maturity. From selected grains taken in order of arrested development a complete series can be constructed. In fact, such a series can often be found in a single anther (plate 3, A, C, H, I). However, by far the larger number abort before division of the microspore nucleus. Since the one-nucleate grains may persist as late as the time of maturity of normal grains abortion takes the form of a delay rather than of disintegration.

A study of the late tetrad, at the time of wall dissolution, in which the microspores are still in position, shows typically an even development. Size differences between the microspores become conspicuous after further growth. In cases of early cytoplasmic abortion in grains no larger than one-half of the mature diameter, the wall undergoes partial or even nearly complete thickening. In others often of greater size, there is less thickening of the exine. The germ pore, however, is formed in all cases where development is carried beyond the normal time of its formation. In general, since there may be wall thickening and enlargement accompanying early cytoplasmic abortion, there does not appear to be an intimate interdependence between wall and cytoplasm although nearly complete wall-thickening is always found in cases in which abortion occurs at a late stage. TISCHLER (1908) has regarded wall formation in pollen as being more or less independent of normal cytoplasmic development. This interpretation would appear justified in view of the condition found in pollen before dehiscence, in which some aborted grains are nearly devoid of stainable cytoplasm and in others only the broken down remnants of the nucleus and cytoplasm remain. The fact

however, that thickening in the wall takes place so early in growth, before degenerative processes are complete suggests that wall thickening is dependent upon cytoplasmic growth. This view is further supported by the thin walls found in those few grains in which abortion occurs before wall thickening.

Considering now the condition of the chromatin in the aborted series, those grains in which abortion occurs at the earliest stages show but little if any increase in amount in the stainable cytoplasm over that received from the mother-cell. In such cases the chromatin still persists in large masses, a condition which suggests only partial reorganization. The nuclear membrane in such cases may be either compressed and irregular in outline or very much extended. The cytoplasm has different reactions to stains, in some instances being finely granular and flocculent and in others dense and more homogeneous. In general those grains which have developed no farther than the one-nucleate stage are characterized by their small size, scant cytoplasm, large vacuole, irregular nuclear membrane, and large chromatin masses. Yet in these there are generally thick walls and normal-appearing germ pores.

Where development is carried as far as the division of the microspore nucleus, there is in most cases considerable addition to the cytoplasm, but the nuclei show a similar condition to that above described for the single nucleus in that there is a suggestion of arrested reorganization as shown by the condition of the chromatin. The cytoplasm may still show a large vacuole or the stainable cytoplasm may fill up the entire grain, in the latter case the staining reaction is lighter (plate 3, J, K). This condition in the cytoplasm indicates abortion at later stages, in which cases the nuclei may appear more normal and may even enter the contracted stage. It is probable that the latest cases of abortion cannot be detected by the appearance in stained sections but are shown by the inability of the grain to send out tubes. It has been stated in the discussion on pollen development that on the stigma some apparently normal grains do not send out tubes. It is conceivable that in these grains which do not develop tubes the end of the aborted series is to be found.

Since the development of pollen is so typically normal up to the time of liberation of the microspore, it now becomes important to determine whether the other elements of the anther show a normal growth, especially in anthers where there is a large percentage or even total pollen abortion. A careful study of anther development in a large number of forms shows that the anthers undergo the usual differentiation even when the pollen they bear is completely aborted. The exceptions to

this are found where there is complete abortion early in development, in which case there is often an unusual ingrowth of the endothecium. Even in these cases the mature anther is filled with sap and the tetrad wall and tapetum completely disappear.

The general development of the different elements of the anther and especially a normal sequence of development in anthers where only a part of the pollen is aborted would appear to eliminate any influence from this source as a factor in pollen abortion. The abortion of some grains in the same substratum in which others not only develop normally but function normally indicates differences between the grains rather than localized influences from the anther.

In view of the condition in the anther, where there is approximately a normal development independent of the pollen condition, it is of interest to find the microspore wall undergoing a more or less independent development. It will be seen therefore that so far as the $2x$ tissue is concerned abnormalities which might later influence pollen development do not enter and that abortion begins with the $1x$ condition.

Earliest evidence of pollen abortion

It has been emphasized that typically abortion does not become evident until after microspore liberation. However, certain apparent exceptions to this in an extreme hybrid condition have been found. In a cross between *P. Besseyi* and *P. armenica* the stages of the heterotypic and homoeotypic division have been studied in detail and there are indications in some of the mother-cells of irregularities in chromatin distribution, which indicate that processes resulting in abortion begin earlier in some cases than liberation, which in the less radical crosses is the typical condition. In this cross 87 percent of the pollen is aborted (table 4).

The earliest indication of arrested development found is in the dyad stage (plate 2, 12) although at the heterotypic division single chromosomes sometimes lie far to one side of the plate and at the early metaphase they are even more scattered (plate 2, 13). This scattered condition of the chromosomes is suggestive, especially in view of the condition at later stages. In a number of the dyads the chromosomes of one nucleus were at late metaphase at the time of complete reorganization of the sister nucleus. In other mother-cells of this cross at the tetrad stage, the conspicuous rings or circles in the cytoplasm (plate 2, 14) which resemble small nuclei in some instances, particularly after the heterotypic division,

also indicates an unbalanced condition following the reduction divisions. As many as thirteen of these rings have been counted in a single tetrad. Some of the darker-staining bodies in the cytoplasm have every appearance of being chromosomes which have not entered into nuclear reorganization. That the rings, or in some instances spheres, which appear so conspicuous in the cytoplasm (plate 2, 15, 16) are connected with chromatin distribution and are evidences of early degeneration is supported by the extreme condition found in such tetrads as that shown in figure 16, plate 2. In the tetrad illustrated here no nuclei have been formed and the rings and dark-staining bodies are conspicuous features in the cytoplasm. Two of the spherical masses may in fact be interpreted as nucleoli.

Following the heterotypic division some abnormalities are found in nuclear reorganization. The variations found at this time include the formation of as many as three nuclei (plate 2, 18) in the place of one, or rarely, the organization of one large and one small nucleus in a single microspore. Sometimes an unusually large nucleus is formed somewhat in advance of the others. Following the liberation from the tetrad wall, the unusually small microspores sometimes found (plate 2, 19) appear to complete the series of variations from the condition found in most of the other forms. While stages earlier than these—from diakinesis through to the end of the heterotypic division—have been studied, there are no outstanding conditions which would justify placing the beginning of degeneration earlier than the period of reorganization of the dyad nuclei—and degeneration at this stage is extremely rare.

Evidence of irregular chromatin distribution in the division of the mother-cells in hybrids has been reported by JUEL (1900), TISCHLER (1908), ROSENBERG (1909), LEVINE (1916) and others. This condition is of interest here primarily in that it does not result in a type of pollen abortion different from those more nearly normal cases where it has not been found. It appears, however, that the condition found at nuclear reorganization following the heterotypic division justifies the conclusion that degeneration processes may begin earlier in the plum than in *Vitis* or *Fragaria*.

The breaking up of pollen into globules

In addition to the processes resulting in the aborted-pollen series, there enters another process which dissolves pollen. This dissolution of pollen results in the production of a yellowish oily mass, which makes the pollen of some varieties sticky and therefore less easily blown away by

wind. This condition is of wide occurrence in the plum, particularly where a large percentage of the pollen is aborted. All stages in the formation of this yellowish substance have been studied and it is found that the substance is made up primarily from pollen although the tapetum may also enter into its formation.

In the process of dissolution the pollen walls are first affected. Some stages in cross-section show a beaded condition of the wall, a bead or globule being formed at each ridge in the exine. In the final stages the wall may be intact, yet completely composed of small globules which gradually merge into larger ones. The small globules of the earlier stages are formed from a relatively thin wall which determines their diameter (plate 3, E). Before the exine breaks up into globules a thick homogeneous band similar in appearance to the tetrad wall is sometimes formed about the cytoplasm. Whether or not this is due to a thickening of the intine could not be determined. At the time of breaking down of the exine into globules, the cytoplasm shows a more diffused staining reaction. Later stages show some globules as large as the mature pollen grain which may further merge into even larger masses, but the final stages usually show a number of smaller globules yet separate (plate 3, D). The tapetum sometimes is affected in the same way as the pollen although it usually functions normally. This dissolution process is general in the loculus although it more commonly affects aborted pollen. It is found in some cases to affect grains with apparently normal nuclei and occurs in pure forms as well as in hybrids. This dissolution process, however, does not necessarily take place, but when it does it is found to occur most frequently near the time of maturity rather than at an earlier stage in development. There is also considerable variation in the extent of dissolution in the anthers of a single flower. Since it does not always take place even in aborted pollen, dissolution does not necessarily appear to be the final process in abortion but rather a supplementary process—undoubtedly enzymatic in nature.

Pistillody and petalody

So far pollen abortion has been discussed aside from the occurrence of any metamorphosis in the anther. There is a wide-spread tendency in the plum for stamens to change into petals on the one hand and into pistils on the other. These abnormal types, including acalycine flowers, have been noted previously by WAUGH (1896, 1900) and others. In the course of metamorphism into these organs an elaborate series of intermediate forms occurs.

In pistillody (plate 4, C, D) particularly, the usual ontogeny of stamen development is upset to such an extent that the following series can be constructed showing the variation in the degree of development of different organs: (a) The nucellar tissue is developed normally in some pistilloids, and not at all in others; (b) the integuments vary from an entire enclosure of the nucellus to complete absence; (c) the position of the ovules in the pistilloids ranges from a terminal position near the anther to a basal position; (d) there are all degrees of enclosure of the ovules by the carpel wall; (e) there are all degrees of stigma and style development; (f) there are all stages in embryo-sac growth up to normal embryo-sac development; and finally (g) in anther suppression there is a great variety of odd outgrowths of stigma and anther tissue terminating the filament-like style. Some of these stigmas become receptive and in some the ovules swell. However, it is important to note that pistilloids are borne in the position of stamens, and hence on account of the abscission of the calyx tube do not persist long enough for fertilization to occur or for the style to be cut off. Even the extreme metamorphosis into an apparently normal pistil does not prevent the shedding of the calyx tube at the usual time or interfere with the usual functioning of the central or normal pistil.

Petalody like pistillody also occurs frequently, and all stages of metamorphosis of an anther into a petal are found (plate 4, A, B). The yellow anther tissue assumes various shapes and sizes as the transition to the white tissue of the petal becomes complete. Yellowish borders of anther tissue generally develop in the narrower petaloids. The tissue of the filament is very similar in external appearance to that of the petal and as with the style, the lateral wing-like appendages lead up to the broadened end which may be part anther and part petal. The most common type of anther bearing the white tissue of the petal is shown in plate 4, A, in which the end is pointed and white. There is little relation between the broadening of the petaloid and the suppression of anther tissue, since the latter may be absent on either narrow or broad petaloids. However, transformation has not gone so far in the case of the petaloids as to form an abscission layer at the base as in the petal, and all petaloids which were observed were found to be shed, like stamens, with the calyx rather than with the petals.

Sections through petaloids taken from flowers at full bloom show a variety of unusual shapes in the loculi. Some are long and narrow, others wide with irregular outlines; still others are lobed or branched, tapering in some to long points, which are devoid of any trace of re-

productive tissue other than an occasional collapsed mass of cell walls. Petaloids also show great differences in the extent of pollen development; in the opposite end of a loculus bearing normal grains, pollen development may have been stopped as early as at the stage of the liberated microspore.

In pistillody and petalody three things are outstanding: (a) in the most extreme instances of metamorphosis the manner of abscission of petaloid or pistiloid is the same as that of the normal stamen, viz., with the calyx tube; (b) these metamorphic changes may result in complete abortion; and (c) they have not introduced a new type of abortion from that found where anther changes in these two directions are not taking place.

An explanation of the phenomena shown in these changes is not easy. The departure from normal growth takes place in somatic tissue first, and if growth is carried far enough, normal-appearing pollen and embryo sacs are produced. This would lead to the inference that the factors upsetting normal development are sporophytic. Something of the nature and extent of the changes which take place when petalody reaches the double-flowered commercial types is shown by its inheritance, as in stocks in which doubleness is dominant to singleness. It is probable that some of the pistiloids would actually set fruit if they were not cut off so early by the dropping of the calyx-tube. There does not appear to be any great difference, except in position, between pistiloids borne in the position of stamens and accessory pistils borne about the primary central one. In those cases in which more than one pistil has been found, pistiloids are not necessarily formed. In fact, in a peach hybrid which bears occasionally as many as five pistils and frequently three, the conspicuous feature of the flower is its doubleness. But doubleness in some of the ornamental flowering varieties of *Prunus* does not interfere with the growth of a normal pistil. So, while pistillody and petalody take on various strange forms in this genus, they appear to be due to forces which do not materially modify the aborted pollen types or interfere markedly with the production of a normal pistil, even though many other pistils may be produced in varying degrees.

The extent of aborted pollen

The extent of pollen abortion in selected forms has been determined partly from mounts in lactic acid and partly from stained preparations. The results are presented in tables 3 and 4.

In table 3 the individuals of two crosses between *P. triflora* and *P.*

americana mollis are classified according to the percentage of aborted pollen found. It will be noted that two varieties of *P. triflora*,—Abundance and Burbank,—have been used as the pistillate parent. In determining the extent of abortion in these hybrids an average of 425 grains was counted in each individual in the Abundance \times Wolf crosses and 730 in each of Burbank \times Wolf crosses. The percentages based upon such large numbers undoubtedly represent the pollen condition fairly ac-

TABLE 3

The aborted pollen in the F_1 individuals of the interspecific cross, *Prunus triflora* (Abundance and Burbank) \times *P. americana mollis* (Wolf).

	Number of trees	Percentage of aborted pollen in the F ₁ plants																	
		5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	
Abundance × Wolf..	34	2	3	4	9	4	5	2	1	2	1		1						
Burbank × Wolf....	23	4	1	3	1	2	3	3	1			2		1		1		1	

curately. In Burbank 32 grains were aborted in 1003 and in Wolf 52 in 226 (table 4), the condition not being determined in Abundance. It will be seen that aborted pollen is present in large quantities in the F_1 progeny of each cross, and also that there is a slightly greater range in the abortion in the Burbank crosses.

From table 4 it is apparent that extreme hybrids in the genus *Prunus* are unable to complete the development of large proportions of pollen—a condition which also obtains in monospecific varieties and forms generally regarded as pure species. In fact, the degree of pollen abortion may lead to a question of the purity of some of the so-called pure forms included in the list. There is some variation shown in the amount of defective pollen between individual trees of a clone; this point, however, was not checked extensively.

The general pollen condition of the forms listed as pure species contrasted with that of the hybrids is shown in table 5, in which each form is classified according to the percentage of pollen aborted. In this way the greater amount of defective pollen in the hybrids is emphasized.

The pollen condition in the plum may be briefly summarized as follows: Abortion occurs for the most part between the time of liberation of the microspore from the tetrad and maturity—in other words during the gametophyte generation. The fact that anther development is normal even when there is a larger percentage of, or complete, pollen abor-

TABLE 4

The pollen condition in certain species and selected varieties of *Prunus* in which a number of interspecific crosses are included.

Variety	Total number	Number normal	Number aborted	Percent aborted
Aitkin (<i>Prunus nigra</i>).....	214	167	47	22.0
<i>Amygdalus nana</i> × <i>P. persica</i>	327	51	276	84.4
Blush (<i>P. americana</i>).....	216	190	26	12.0
Burbank (<i>P. triflora</i>).....	1003	971	32	3.2
Cheney (<i>P. nigra</i>).....	220	101	119	54.1
Compass (<i>P. Besseyi</i> × <i>P. hortulana</i> Mineri)	214	121	93	43.5
“ “ “	—	—	—	100.0
“ “ “	211	90	121	57.3
De Soto (<i>P. americana</i>).....	226	209	17	7.5
Etopa (<i>P. Besseyi</i> × <i>P. triflora</i>).....	200	152	48	24.0
“ “ “	207	182	25	12.1
Ironclad (<i>P. americana</i>).....	211	181	30	14.2
“ “ “	257	235	22	8.6
“ “ “	232	209	23	9.9
Loring (<i>P. triflora</i> × <i>P. americana</i> ?).....	—	—	—	26.0
Manitoba (<i>P. nigra</i>).....	214	183	31	14.5
Ocheeda (<i>P. americana</i>).....	315	295	20	6.3
“ “ “	372	265	107	28.8
Opata (<i>P. Besseyi</i> × (<i>P. Munsoniana</i> × <i>P. triflora</i>)) (plate 3, B, F).....	200	40	160	80.0
<i>P. americana</i> (wild).....	302	237	65	21.5
“ “ “	228	195	33	14.5
“ “ “	214	185	29	14.0
<i>P. angustifolia</i>	226	185	41	18.1
<i>P. Besseyi</i>	211	185	26	12.3
<i>P. Besseyi</i> × <i>P. americana</i>	353	128	225	63.7
<i>P. Besseyi</i> × <i>P. armenica</i>	225	28	197	87.6
“ “ “	209	37	172	82.3
“ × <i>P. Simoni</i>	211	137	74	35.1
<i>P. nigra</i>	237	180	57	24.1
“ “ “	206	128	78	37.9
“ “ “	222	214	8	3.6
<i>P. pennsylvanica</i>	436	321	115	26.4
<i>P. virginiana</i> (plate 3, L).....	233	198	35	15.0
“ “ “	273	253	20	7.3
Rollingstone (<i>P. americana</i>).....	392	254	38	9.7
Sapa (<i>P. Besseyi</i> × <i>P. triflora</i>).....	355	206	149	42.0
Surprise (<i>P. hortulana</i> Mineri).....	309	204	105	34.0
“ “ “	200	186	14	7.0
Wolf (<i>P. americana mollis</i>).....	226	174	52	23.0
Wyant (<i>P. americana</i>).....	248	207	41	16.5
“ “ “	187	153	34	18.2
Yellow Egg (<i>P. domestica</i>).....	510	494	16	3.1

TABLE 5

A classification of the hybrids and pure forms listed in table 4 on the basis of the percentage of aborted pollen.

	Percentage of aborted pollen																			
	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
Hybrids	1	1			2		2	1	1		1		1				2	1	1	1
Pure forms	6	5	6	4	3	1		1			1									

tion, tends to eliminate conditions in the anther as a constant factor in abortion. In extreme hybrid forms there is evidence of pollen disintegration as early as the dyad stage. The breaking down of pollen into yellowish globules appears to be a supplementary enzymatic process separate and distinct from true abortion, since it does not always occur and sometimes affects mature grains which have every appearance of being normal. A new type of aborted pollen is not introduced by pistillody and petalody. Neither have these metamorphic processes been found to affect the normal method of stamen dehiscence. Aborted pollen occurs in the plum in considerable quantities, even in many so-called pure species, but is not sufficient in these, considering self- or cross-pollination, except in a few extreme cases of complete abortion, to be of itself a prohibitive factor in the setting of fruit. However, in view of the extreme hybrid condition of many varieties, aborted pollen in them becomes of greater importance. The abortion of pollen during the haploid stage appears to point to a state or condition in the germplasm of the gametophyte as the cause of abortion.

PISTIL DEVELOPMENT IN RELATION TO STERILITY

In the section on pollen development it was shown that self-sterility and cross-sterility as well, are of the type generally referred to as incompatible and are not necessarily due to defective pollen except in extreme cases of complete abortion. It now remains to determine the factors in pistil development which enter into the general question of sterility. As in the case of the pollen, the course of normal development will be presented first.

Pistil development

Early stages

The degree of development of the pistil in late winter is shown in figure 20, plate 2. There is as yet no protrusion of the growing-point

from which the ovule will develop. It will be seen that the carpel cavity is formed by the folding together of two margins which do not unite until considerable growth has taken place. The point of union forms the suture which is clearly distinguishable either at bloom or at maturity. The two ovules are borne on a parietal placenta close to but on either side of the suture. The stigmatic cells at this time appear no different from the other cells of the epidermis. The pistil, then, in the winter bud is rudimentary and its special structures are not formed until the early spring growth.

The pistil at bloom

In the treatment of weather in relation to fruitfulness (DORSEY 1919) the principal factors affecting receptiveness, namely, the stigmatic surface and the abscission of the style were discussed. These will not be dealt with further here, since, in the light of the evidence to be presented later, on the failure of so many pistils to set fruit, interest centers primarily about development in the ovule.

In the course of this investigation sections have been made of pistils at various stages of growth from a large number of different varieties and species. While some of the variations found in *Prunus* species have not been noted by PÉCHOUTRE (1902), his studies of pistil development in the Rosaceae in general and particularly in *Prunus* have been so thorough and are presented in such detail that further treatment in this connection would be largely repetition. The following discussion of the mature pistil has been carefully checked with that of PÉCHOUTRE in species available in this investigation.

At the time the flowers open, the embryo sac may contain from one to eight nuclei, although generally there are but two or four. The embryo sac increases in size but little before fertilization. The nucellus is made up of large, thin-walled cells with a "cap" of smaller, thicker-walled cells at the apex. The two integuments are concrescent at the chalaza but otherwise separate throughout their length. The outer integument is five cell layers in thickness and epidermal in origin; the inner is three cells thick and sub-epidermal in origin (PÉCHOUTRE 1902). The distal ends of the integuments, especially of the outer, make a very irregular growth and push well up into the narrow end of the carpel (plate 2, 21; and plate 5, B). This extension of the integuments forms a large canal or channel through which the growth of the pollen tube is tortuous. Tubes frequently miss the opening to this channel and grow downward in the

cavity of the ovary but none has been found to enter the chalaza. At the distal end of the carpel cavity there is an irregular ingrowth of tissue—called the “obturator” by PÉCHOUTRE—which partly projects over the micropyle (plate 2, 21, a). The anatropous ovules gradually assume an upright position and at the time of fertilization are borne upright on a parietal placenta.

In external appearance pistils vary in many features. In *Prunus domestica* the style is much thicker and much more pubescent than in *P. americana*, and the characteristic green color is partly obscured by a purplish pigment in *P. pissardi*. The suture also is deeper and more distinct in some varieties than in others. In all species studied, however, the cells of the central core in the style have much denser cytoplasm than have the cells of the surrounding tissue (plate 5, I., a), and no tube growth has been found outside toward the epidermis. The tubes pass between cells by dissolving the middle lamella, since, with the exception of a few cases in the suture for short distances below the stigma there is no canal in the style of the plum. At the first the course of the tube is very tortuous, but farther down the style it is more direct, the tube is narrower, and its stainable portion is longer. Vacuoles soon appear in the cytoplasm of the cells of the central core near the tubes; and later, before abscission all cells of the central core are more or less vacuolized. The point of abscission is much higher in some species than in others—in fact “pointed” plums are generally characterized by the persistence of a stub of the style.

The ripe seed

After fertilization there is great variation in the degree of development in the embryos in different pistils but there is a very rapid extension of the embryo sac into the canal. Coincident with embryo-sac extension there is rapid growth in the endosperm whose free nuclei form a thin jacket one cell layer thick around the inner surface of the embryo sac. Walls do not form between the nuclei of the endosperm for some time after the complete extension of the embryo sac to the chalaza. As the embryo sac enlarges, a conspicuous channel is formed in the nucellus, which is completely absorbed during the period of rapid endosperm growth. In many sections the nucellus is broken down considerably in advance of the embryo sac. After the endosperm is formed, it, like the nucellus, is largely absorbed and gives way very rapidly to the growing cotyledons. The endosperm, however, is never completely absorbed in

this genus and can still be found in the ripe seed in irregular patches between the cotyledons and the seed coat, to which latter it is always joined and of which it may be considered to form a part (plate 2, 22, a). This condition led PÉCHOUTRE to suggest that the Rosaceae arose from plants with seed albumen.

The "pit" in the plum (plate 2, 22, b) is formed by the laying down of stony tissue about the inner border of the carpel. The ovary wall thickens to form the fleshy edible portion of the ripe fruit. Hardening in the stone cells does not take place until the normal size of the pit is reached. Since typically only one embryo is matured in each pit, it remains to be seen what disposal is made of the other one which is always found in the early stages.

The suppression of one ovule

During the earliest stages of floral development no differences are evident in the two growing points on the placenta. At bloom there may still be an even development of the two ovules (plate 5, A) and rarely two embryos may develop to maturity (plate 4, H) in the same "pit." Such development, however, is not the typical condition and while abortion is found at all stages between the megaspore mother-cell and maturity, in most varieties one ovule shows an arrested development previous to fertilization. After fertilization the smaller ovule is quickly surpassed by the other and in the mature seed only the brown remnants of the integuments persist (plate 5, D).

In the earliest stages of arrested growth found in the suppressed embryo, the integuments are normal in appearance and degeneration begins first in the megaspore and the cells of the nucellus immediately surrounding it. In some of the sections of the earliest stages the nucellus is a degenerate mass and the integuments are partly drawn together (plate 5, C). In these early cases of suppression the ovule turns brown and there is no further growth. In most instances, however, the embryo sac is formed. If both ovules develop normal embryo sacs, the one in which fertilization takes place first apparently gains the ascendancy. Following fertilization, size differences soon become pronounced and in those in which fertilization does not take place the liquid is completely withdrawn. If fertilization takes place in both ovules, which sometimes happens, suppression takes the form of embryo abortion, in which case in the ripe seed the coats are often devoid of contents except for the partially developed embryo.

There is a great difference between varieties in the degree of development of a second ovule. The relative size of the ovule at the time of bloom was studied by outline drawings, and these show an equal development in a large number of pairs in some varieties and a large number of cases in other varieties in which one ovule is conspicuously larger than the other. The drawings show, however, that neither ovule is constantly larger than the other. Near maturity, the greatest development in the suppressed ovule was found in *Stella*, a cross between *P. triflora* and *P. americana*. A series in the growth of the second embryo in this variety is shown in figure I, plate 4. This is in marked contrast to *Asiniboin* (plate 4, J) taken at approximately the same stage of development.

Thus it will be seen that the typical condition is for one embryo to develop in each pit. In other words, approximately one-half of all ovules are suppressed, although normal development in one is all that is required for fruit formation. This condition prevails in varieties representing a single species or in extreme interspecific crosses and it occurs alike in varieties bearing normal pollen and in varieties in which most or all of the pollen is aborted.

The dropping of pistils

Under orchard conditions pistils fall at three distinct stages: (a) at or immediately after bloom, (b) from two to four weeks after bloom, and (c) later, following considerable enlargement in the pistil. For convenience in presentation these will be taken up in the order of their occurrence.

The first drop

The extent of pistil abortion has been studied by a number of investigators, and the general conditions reported are comparable to those found in Minnesota. BAILEY (1892) reports finding a wild tree of *Prunus americana* which "bears flowers without pistils." LORD (1894) found that all varieties sometimes bear flowers with aborted pistils. HEIDEMANN (1895) found 90 percent of aborted pistils in *Hiawatha*. GOFF (1894, 1895) determined the degree of pistil abortion in a number of varieties of *P. americana*, which in some cases, as in *Moreman*, amounted to as much as 74 percent. PETERS (1916) evidently does not recognize dropping from aborted pistils and ascribes the falling of flowers "a few days after the petals" to non-pollination. WAUGH (1896)

found 100 percent "defective pistils" on several trees. He considers that in the aggregate they are "numerous enough to be taken into serious consideration," but that they do not exert an influence on the crop "except in uncommon cases of total defectiveness." In a later publication, WAUGH (1897) presents extensive data showing the percentage of defective pistils in a number of varieties in nine plum groups. Defective pistils have been given considerable attention in the present investigation, but the partial summary of WAUGH's data given in table 6 expresses the general condition very well. It will be seen that defective or aborted pistils are of wide occurrence and of sufficient numbers in many instances to have an influence upon the crop.

TABLE 6

A summary of the data collected by WAUGH (1897) on the extent of pistil abortion in the plum.

Group	Percentage of defective pistils																				No. of varieties	Average per centage for group	
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95			100
<i>Prunus domestica</i>	69	16	12	3	2		5		1	1												29	4.3
Japanese	34	7	10	7	3	2		2		1	2		1				1					22	11.2
<i>P. americana</i>	59	20	16	11	13	9	11	6	8	2	5	4	3	2				1		6	4	55	21.2
<i>P. nigra</i>	9	4	3	1	2	1	1	1	1	2												6	17.0
Minor	17	7	3		2																	10	1.9
Wayland	14	3	5	5	2	2	2	1	1	1							1		1			12	10.5
Wildgoose	22	3	8	5	4	2		3	2			1		1	1				1		1	18	19.8
Chicasaw	24	11	8	4	2	2	2	2	3	2	3		1	1			2	2	1			20	10.5

Pistil abortion occurs at any stage from that of a slight growth of the flower rudiments in the early spring to the time at which the flowers have reached normal size at bloom; but for each variety the degree of growth reached before abortion is quite characteristic and varies but slightly. Flowers with the earliest-aborted pistils drop first but always come into full bloom. When abortion occurs so late that the pistil reaches normal size, but little further growth takes place and dropping may occur as late as a week after bloom. Flowers with defective pistils always drop at the pedicel base and neither the calyx tube nor style is shed by abscission because growth is not carried far enough. Pistils aborted at the earliest stages turn brown or black while the later ones to abort are distinctly yellow compared with the green of normal ones. Two features, therefore, characterize this drop: (a) the flowers bear aborted pistils, and (b) falling takes place soon after bloom. The immediate cause of the dropping of the flower appears to be pistil abortion.

Sections have been made of a large number of these defective pistils,

and in all cases *both* ovules are found to be aborted (plate 5, E, F). The aborted series presented is comparable with that of the single ovule previously discussed. In the larger aborted pistils the dark color of the degenerated ovules can be seen distinctly through the carpel wall. In the earliest cases of abortion found the size of the pistil was several times that of the pistil in the dormant winter bud. It is of interest to note also that pistil and ovule abortion have no apparent influence upon pollen development.

The early abortion of pistils has been assigned to various causes. GOFF (1901) held that abortion is due "in the majority of cases to a return of cold weather" after spring growth has once started. WAUGH (1896) determined the percentage of defective pistils from nine varieties at different points between Denison, Texas, and Ottawa, Canada, which do not sustain the conjecture of GOFF because of the large numbers which he found aborted at all places.

The fact that some varieties bear fruit in abundance for a number of years, and afterward for one or more seasons suddenly produce flowers that show a complete abortion of pistils suggests a definite relation of this condition to nutrition. Observation of changes of this nature have been made by a number of investigators (LORD 1894, 1899; GOFF 1894, and others), and two striking instances have occurred at the Minnesota Station. One variety, Wickson, bore two heavy crops of crossed plums in the greenhouse, and the following year all pistils were aborted. In the second instance, Wolf under orchard conditions bore heavily in 1914, and for three consecutive seasons afterward produced less than 1 percent of normal pistils. Wickson was not subjected to killing temperatures previous to the time of abortion.

The occurrence of aborted pistils in varieties of so many different species under cultivation as well as in wild seedlings indicates that the tendency to the appearance of this condition, whatever its cause, is widespread. The occurrence of so many aborted pistils in seasons following heavy fruits suggests, as noted, a connection with nutrition. It is a matter of general observation among fruit growers that heavy fruiting is likely to be followed by a light crop, but under these conditions fruit-bud formation is usually reduced. While the relation between food supply and fruitfulness has not been definitely explained as yet on experimental grounds, the general relation shown in the orchard justifies placing some confidence in the assumption of the relation between aborted pistils and nutrition. If this assumption is correct, a small degree of abortion may be interpreted as suggesting a competition between differ-

ent flowers in a bud while a total abortion indicates a much more general condition.

The earliest stages of pistil abortion have been given careful cytological study. The first appearance of disintegration is found in the ovule and consists of a breaking down in the embryo sac. The disintegration stages here closely resemble in general details those already discussed in the abortion of one ovule. In the aborted-pistils series both ovules are affected; in fact, the general suppression of one ovule may be regarded as the first step in the aborted series and needs only the suppression of the other to complete it. When this condition is taken into consideration, together with the fact that early and late abortion take place, the series is complete. This indicates a condition of instability in the pistils in this genus, which is suggestive of dioeciousness has not yet, as will be shown later, progressed so far as to show a constant morphological difference between flowers.

The second drop

The first drop is followed two weeks or so after bloom by another distinct wave of falling pistils. While there are a few intergrading forms between these two drops, certain features of the second drop separate it distinctly from the first.

Unlike the pistils of the first drop, those of the second have every external appearance of being normal. Enlargement up to a certain point takes place and in most cases the calyx tube breaks away at least in part even though there is insufficient growth in the young plum to throw it off. The style is not deciduous in the earliest pistils to fall, but, like the calyx tube, drops in those which fall later (plate 1, D). In *P. Besseyi*, however, both the style and the calyx tube persist longer than in other species (plate 1, C). Pistils which fall in the second drop, as in the first, abscise at the pedicel base while the pistil is still green, although the pedicel has become light yellow. Yet in the last pistils of the second drop to fall the abscission layer is formed at the base of the ovary (plate 5, K) and in some instances can be easily broken off at this point. Even the last pistils to fall, which are usually the larger ones, are still turgid, and while abscission generally precedes browning and shriveling, in some varieties the integuments turn brown and the sap of the nucellus is partly or completely withdrawn in many of the pistils before they fall. The browning appears first in the chalaza and gradually extends to the nucellus and integuments and sometimes even to the stone tissue.

From the foregoing it is evident that there are characteristic features which distinguish the second drop from the first.

By referring to table 7, it will be seen that at about three weeks after bloom in accounting for all the flower buds or flowers produced by a tree only four different categories are necessary. Other varieties could have been included in the table but these four illustrate something of the extremes encountered. These counts were made in the spring of 1917.

TABLE 7

A percentage classification of the total number of flowers borne by four varieties of plums, on the basis of those which were winter-killed, those which fell at the first drop, at the second drop, and those which "set."

Variety	Date of bloom	Flower buds winter-killed	First drop	Second drop	Number set
Assiniboin	May 17	0	34	55	11
Minnesota No. 18 ¹	" 21	10	50	35	5
" " 21 ²	" 19	42	46	7	5
" " 35 ¹	" 19	15	21	54	10

¹ Abundance \times Wolf.

² Burbank \times Wolf.

After deducting the number of flowers winter-killed, those which fell with aborted pistils, and those which set, a considerable number, varying from 7 to 55 percent, remains. These constitute the second drop, which in number is comparable to those of the first drop. In fact, it will be seen that in one variety the first drop may be greatest and in another the second, and that in all cases the number of pistils listed under either drop is greater than the percentage to set. Leaving out of consideration the flower buds killed during the winter and those eliminated in the first drop, as a rule there still remains a sufficient number to produce more than a crop if fertilization occurs in them. These are analyzed further in table 8 in order to determine what size differences occur between those which set and those which drop.

This analysis was begun seventeen days after bloom, when the second drop had started. The size of those dropping and those still persisting (table 8) was compiled from records made in 1917, in which the pistils in each category are put into classes according to their greatest diameter parallel with the suture. Attention is called to the relative size at the time of falling of pistils open to cross-pollination and those from which the stigma was snipped before pollination.

Emphasis is placed upon the following points brought out in table 8: (a) the period of abscission of the second drop extended from 17 to 30 days after bloom; (b) beginning with the first pistils to fall, size differences between those persisting and those which fell, gradually increased with time; (c) pistils which fell within the above-mentioned time limit enlarged only up to a certain point; (d) those pistils with the stigmas snipped before pollination, enlarged before falling, to a size comparable with that of those not so treated; and (e) in each variety there was a gradual increase in the size of the pistils which fell off.

The degree of enlargement of certain pistils over others is again shown in plate 1, A and B, in which the extremes in the percentage of pistils to set, 3.1 and 63.9 percent, respectively, are shown. The distinction in size between the first and second groups of pistils to fall is appreciable, since at bloom the normal pistil is only 1.1 to 1.5 mm through the suture diameter. Only a few of this size are recorded in table 8. Plate 1, D, illustrates the difference between those falling last and those setting, in Minnesota No. 21, thirty-one days after bloom. With this analysis, showing so clearly the size distinction between those falling and those persisting, the evidence as to the cause of the falling of the second group will be presented.

The cause of the second drop

The similarity in size at the time of falling of those pistils open to pollination and those whose pollination was prevented by snipping the stigma, would alone appear to justify the conclusion that the second drop is due to non-fertilization. This point, however, was investigated further by two different methods: (a) by excluding pollen, and (b) by a cytological examination of the ovules.

In the experiments in which pollination was prevented, tents were placed over one tree each of Minnesota No. 21, Assiniboin, Wohonka, Wakapa and *Prunus americana*, during the period of bloom. On account of the total self-sterility of these varieties no emasculating was done under the tents. The entire lot of normal pistils under the tents, including branches with snipped stigmas, those self-pollinated and those not pollinated, showed an enlargement similar to those falling with the second drop under orchard conditions and fell with them. But controlled crosses under the tents set in approximately the same proportion as uncovered trees of the same variety adjacent.

As checks to the tented trees in the orchard, two trees (Manitoba and

Yellow Egg) were held under observation in the greenhouse where pollen was definitely excluded from the stigma by emasculation; on these trees the pistils which were not pollinated showed enlargement before falling, similar to those under the tents. Before concluding finally that fertilization has not taken place in pistils which fall in the second drop the state of development in the embryo sac should be determined.

The condition in the ovule has been examined in pistils definitely known not to have been pollinated. These served as a check to those examined from the second drop. In the absence of fertilization an interesting condition is created in the ovule, which shows something of the dependence upon the egg of development in other parts. The contrast will be shown between the fertilized and unfertilized condition.

The general proportions between the different parts of the pistil are maintained in the partial enlargement which takes place in the second drop. The integuments thicken and expand, a condition which is in marked contrast to that usual in the suppressed ovule. The nucellus increases in size, as was noted by BACKHOUSE (1911 a) in self-pollinated pistils which fell "generally within three weeks," but it seldom breaks down before abscission, although there may be a partial withdrawal of sap. The canal as a rule extends into the nucellus but little beyond the chalazal end of the embryo sac in the pistils which fall earliest in the second drop; later, however, there is considerable canal extension. In one pistil from which the stigma was snipped before pollination, at thirty-four days after bloom the canal through the nucellus had extended to the chalaza (plate 5, J). This ovule had grown to the largest size of any found in the absence of fertilization and was 4.4 mm long and 1.5 mm wide. PETERS (1916), working with varieties of *Prunus domestica*, states that when self-sterile varieties are self-pollinated, "the carpel swells up to the size of a culinary pea before it falls," but that if the flowers are not pollinated, they fall "a few days after the petals." Apparently he fails to make a distinction between the first and second drop. The point is that ovule development reaches only a certain size in the absence of fertilization, but the pistil in which fertilization has not occurred persists after the style and calyx tube have fallen.

Turning now to the embryo sac, the early stages after bloom where fertilization has been prevented show the normal nuclear condition. Breaking down in the nuclei of the embryo sac first appears from two to three weeks after bloom. Disintegration takes place in the antipodal nuclei first, then in the endosperm nuclei and lastly in the egg nucleus. In figures 25 and 26, plate 2, the first stages of disintegration are shown

in the egg, twelve days after bloom. More advanced stages are shown in figures 23 and 24, plate 2, which are drawn from pistils collected 24 and 34 days respectively after bloom. In the last instance pollination was prevented by snipping the stigma. It will be seen that the nuclei in the embryo sac persist long enough to allow for a considerable delay in pollination. The persistence of the egg in the absence of fertilization is decidedly different from the condition in the apple, in which KNIGHT (1917) found that "at 120 hours the egg cell begins to show disintegration."

The embryo sac elongates but little if fertilization does not take place (plate 5, G, H). Even in the case noted above of exceptional ovule development there was but very slight elongation of the embryo sac. Cases of this kind suggest that canal extension is independent of the embryo-sac growth when certain sizes are reached in the ovule. Endosperm in the absence of an embryo has been found only in two cases in Yellow Egg (*P. domestica*), in which a few divisions had apparently taken place. As a single exception, an embryo four cells across was found in a pistil falling within the size limits of the second wave of dropping. These instances may be regarded as intermediate between the second wave and the third.

The condition found in the unfertilized series is in marked contrast with that found when fertilization takes place. As early as 18 days after bloom the embryo sac in which the egg has been fertilized extends the entire length of the nucellus to the chalaza, and a jacket of endosperm, usually only one cell thick, covers the entire area of the "dumb-bell-shaped" sac. With the completion of these changes in the embryo sac the embryo may be no larger than four cells across (plate 2, 27). The slow growth of the embryo in the early stages and the extremely rapid formation of endosperm have been emphasized by PÉCHOUTRE (1902).

It will be seen from the above observations that all the evidence shows that fertilization has not occurred in the pistils which fall at the second drop. This is in accord with the statement of WAUGH (1899) that "the germs of the incipient seeds are not fecundated." Pollination may have taken place, but tube growth was retarded to such an extent that fertilization was prevented probably by the abscission of the style. By referring to the article on weather in relation to fruitfulness (DORSEY 1919) it will be seen that this drop can be ascribed primarily to unfavorable weather at bloom—especially rain and low temperatures—which would also account for the differences in the extent of the second drop from year to year.

The third or "June drop"

Following the second drop there is still another—the so-called "June drop." In popular usage the term June drop applies primarily to the third drop of large plums because they are much more conspicuous, but does not include the relatively few which fall from time to time, even up to maturity. WAUGH (1899) distinguishes clearly between the second drop and the June drop. He says that "this first fall of minute fruits (which sometimes takes the whole crop) is commonly supposed to result from non-fecundation of the ovules," but that "this is not the true June drop." BACKHOUSE (1911 a) states that "it seems probable that the trouble known as the June drop of the Americans" is "to be explained as a consequence of self-pollination." According to the evidence presented previously, self-pollinated pistils drop at sizes similar to those at which unpollinated pistils drop and for the same reason. It has been shown that time and size of dropping draw a relatively sharp line between the first and second waves of dropping. Likewise these two factors separate the second drop from the third. From table 8 it will be seen that when fertilization does not take place enlargement reaches only a certain point, the maximum recorded being in the 5.6-6.0 mm class, while the mode is near 3.0 mm. Among the last of the second drop an occasional ovule is found with slight embryo development, which shows that there are connecting forms between the second and third drops as well as between the first and second. In approximately one month (table 8) the second drop is over, and those setting have so increased in size as to place them in a distinct size class from those which have fallen.

The size of plums in the third drop is shown in table 9. By comparison with table 8 it will be seen that considerable enlargement has taken place and that the size of those falling is comparable with the size of those persistent at the earlier date.

The measurements of Assiniboin and Winnipeg were made forty days after bloom and include a small number of fruits which fall without injury and a large number injured by curculio stings. These fell for the most part with the third drop and have generally been considered a part of it. Dropping occurred as late as forty to eighty-three days after bloom. In the cross Compass \times Yellow Egg the entire crop fell just before maturity. So plums which fall at this drop, like those which fall at the first and second, have a characteristic size with a considerable range in the time of falling but with a pronounced mode. It should also

TABLE 9

Showing the size of plums falling at the June drop. Under the headings Assiniboin and Winnipeg the size of those falling from curculio stings is also given.

	Assiniboin	Minn. No. 6 × Manitoba ²	Compass × Yellow Egg	Minn. No. 21 × Burbank ²	Winnipeg
Date	June 26	May 7	June 22	June 21	June 26
Days after bloom	40	54	83	82	40
Size of plums	Dropping Dropping ¹ Persistent	Dropping	Dropping	Dropping	Dropping Dropping ¹ Persistent
5.6-7.5	1		1		
7.6-9.0	2		6		
9.1-10.5	2 1		13		
10.6-12.0	1		25		1 1
12.1-13.5	13		17	2	3
13.6-15.0	25		15		12
15.1-16.5	1 15		2	8	5
16.6-18.0	7			8	3
18.1-19.5	2 2			12	
19.6-21.0				1	1
21.1-22.5				1	2
22.6-24.0		1			
24.1-25.5			3		
25.6-27.0			1		
27.1-28.5					
28.6-30.0					

¹ Stung by curculio.

² Greenhouse cross.

be noted that the method of abscission in the June drop is different from that in either of the others. The conducting tissue of the pedicel, especially at the base, has become hardened, while this condition has as yet not been reached at the base of the ovary. Consequently, instead of parting at the pedicel base as in the first and second drops, abscission takes place between the plum and pedicel and in the most advanced cases, as in the normal falling of the ripe plum, the pedicel does not drop but may persist for one or more years. A further difference is found between the second and third drop in that neither calyx tube nor style is ever present as late as the time of the third drop.

Seed development in the June drop

Sections have been made of the embryos of a large number of plums which fell at the June drop. Dissections were also made of ovules at various stages to determine the amount of growth in the embryo. The general condition found may be summarized as follows: (a) embryo development started but growth stopped at any time from the stage when the embryo was a few cells across to the time at which it had reached nearly the mature size; (b) endosperm had partly formed, but the embryo gained the ascendancy to such an extent that it was often found naked in the nucellus; (c) enlargement in the seed could reach nearly the mature size when fertilization had once occurred, accompanied by only a slight growth of the embryo.

Considerable variation was found in the relative development of embryos. A characteristic of all the seeds dissected in the cross Compass \times Yellow Egg (table 9 and figure E, plate 4) was the small amount of endosperm present. In fact, in some seeds none could be detected. The last stages of the seeds of this cross show a complete absorption of the sap of the nucellus and a drying and withering of the seed coats, within which the embryo appears as a lump at the pointed end. In many of the seeds of this cross neither embryo nor endosperm could be found by hand dissection, indicating an early abortion of both. The canal, however, is very prominent and it is possible that complete embryo-sac extension has taken place before abortion. An early browning of the seed coats soon follows suppression of the embryo. In this cross all plums fell before maturity but after they had reached nearly the normal size.

In a cross between Minnesota No. 21 and Burbank, embryo development differed from that described above. Many plums of nearly mature size—ranging from 15 to 20 mm through the suture diameter—were falling 82 days after bloom. Dissection of the seeds showed considerable growth in the embryo and only traces of endosperm. F, plate 4, is a series showing embryo growth, natural size. Sections were made of some of these embryos, and one of the smaller was only sixteen cells across—yet the plum had persisted for nearly three months. The external appearance of the seeds in this cross is similar to that of Stella (plate 4, G).

It may be argued that fertilization had not taken place in those cases in which an embryo sac could not be found. Even if an embryo could not be found the size of the plum was so much greater before falling than those of the second drop where fertilization is known definitely not

to have taken place, that the size alone may be taken as conclusive evidence of fertilization. This position appears justifiable in view of the fact that in the experiments carried on under the tent and in the greenhouse, in which pollen was definitely excluded from the stigmas, the cases in which the size of pistils approached that of the pistils falling at the third drop were so few as to be negligible.

The great rapidity in endosperm formation and also in embryo-sac extension have been emphasized; therefore the occurrence of embryos without or with only a partial growth of endosperm was not to be expected. The above status of seed development in the Compass \times Yellow Egg cross is not typical. In table 10 some of the variations found in a cross between *P. triflora* and *P. americana* are set forth. The seeds classified in this table were taken from fruit falling just before the normal plums were ripe and were typical of the plums which so often ripen earliest. From I, plate 5, it will be seen that the tissues of the seed are easily distinguished.

TABLE 10

The relative development of the embryos in plums dropping a week to ten days before maturity in a cross between P. triflora and P. americana. All were taken on the same date, August 19, 106 days after bloom and showed no evidence of external injury.

Embryo size	Seed coat	Nucellus	Endosperm	Embryo	Cotyledons
$\frac{3}{8}$	Brown	Sap absorbed	$\frac{1}{4}$ -developed	Present	3 lobes. 8.6 mm long
$\frac{3}{8}$	"	" "	" "	Absent	None
$\frac{1}{2}$	Brown and shriveled	" "	" "	Very small	1.8 mm long
$\frac{1}{2}$	" " "	" "	" "	Absent	None
$\frac{3}{8}$	" " "	" "	Slightly "	"	"
$\frac{1}{2}$	" " "	" "	$\frac{1}{4}$ - "	Present	5 mm long
$\frac{1}{4}$	" " "	" "	" "	Absent	None
$\frac{3}{8}$	" " "	Normal	Normal	Present	12.7 mm long
$\frac{3}{8}$	White and turgid	"	"	"	11.8 " "
$\frac{3}{8}$	Brown " "	"	"	"	11.7 " "
Full size	White " "	"	"	"	14.3 " "

The condition in the later stages of the development shows that there is in each case at least partial growth of the endosperm. The sap of the nucellus is withdrawn in all cases, resulting in the shriveling of the seed coat when the embryo is partially formed. When the embryo is marked "absent" the supposition is that growth stopped early so that it was too small to detect in dissection. In four seeds the endosperm appeared in the irregular patches characteristic of maturity. These plums

were of nearly full size and differed from the others in that they ripened earlier. The condition of seed development near maturity in these was so similar to that found earlier at the June drop that they could, were it not for confusion, be included under that heading.

The status of development in the ovule in the third drop shows marked differences from that in the second. Firstly, a greater size is attained than is ever found in the second drop, and secondly, instead of there being disintegrating nuclei within a slightly elongated embryo sac, tissues cease growing at various stages rather than disintegrating. This latter fact alone suggests an additional stimulus absent in the second drop.

Cause of the June drop

WAUGH (1899) considered that three principal causes enter into the June drop: (a) "non-pollination," (b) "curculio-work," and (c) "the struggle for existence." It has been shown that non-pollination is the cause of the second drop and that the size element alone eliminates this factor from the third drop. After an examination of the aborted embryos in the June drop, WAUGH's conclusion that "it seems fair to conclude that pollination plays a considerable, though varying, part as a co-operating cause of the June drop," appears to be wrong. It may also be stated that although plums stung by curculio fall at this time, that curculio work is not necessarily a cause since it can be controlled and in some seasons is negligible. In addition, the third drop occurs on trees in which the small setting of fruit would not appear to create conditions of competition sufficiently intense to eliminate a large number, and it may also be very slight when there is an exceptionally heavy setting of fruit. This was the condition in one tree of *Prunus americana* in 1918, in which 67 percent of the flowers set fruit (plate 1, B). Growers generally find that the number of fruits set is not reduced to a relatively fixed maximum by the struggle for existence but that the tree matures the excess crop at the expense of size. If the struggle for existence is the primary cause, this drop would be expected to take place later, nearer the time of maturity, when greater demands are made upon the available food.

It will be seen therefore that certain considerations detract from the importance of the struggle for existence as the primary factor in this drop. However, it is not the intention to attempt to eliminate this factor entirely since recent studies (EWART 1907, 1909; MÜLLER-THURGAU 1898, 1908; HEINICKE 1917; and WHIPPLE 1917) show a specialization

and adjustment in the growing parts not heretofore suspected. While the work of the authors cited has dealt primarily with the grape and apple, observations made in this connection show that influences of a similar nature, among them the fruiting habit, are at work in the plum.

Varieties of the different species vary in the production of fruit buds on the terminal one-year growth. Some of the outstanding differences are shown in *P. domestica*, in which only 22 varieties out of 158 bore fruit buds on the terminal annual growth. In *P. triflora* 19 out of 21 bore nearly a full crop. In *P. americana* 18 out of 21 varieties bore fruit buds on the terminal growths. On the other hand, *P. Besseyi* fruits primarily on the terminal twigs.

In the varieties available in this investigation there was a pronounced June drop in the plums borne on the terminal wood. In fact, on the older trees fruit seldom matured in this position. The dropping of fruit from the terminal growths can be partly accounted for on the basis of the competition from a thorn or branch which is developed between the lateral fruit buds on the terminal twigs the second season. This condition occurs over the entire outer area of the tree.

On vigorous six-year-old trees of Minnesota No. 21, an attempt was made to influence the setting of fruit on the terminal growths by removing the flowers from the remainder of the tree. On one tree all flowers were pulled off excepting on the terminal growths, and 53 fruits set on 37 shoots compared with 53 fruits on 55 shoots on an untreated check tree adjacent. The condition on the one-year shoots was not strikingly different from that on the two-year wood of the check on which 23 branches bore 33 fruits. On still another tree the fruiting thorns were cut off, leaving bloom only on the one-year terminal growths. In this case there was a smaller setting of fruit but a very luxuriant terminal growth. It is possible that the treatment noted above was made too late for complete adjustment to take place. All trees of this variety bore a light crop the year of the test,—1917. Under favorable conditions fruit matures on the terminal shoots, but the percentage to set is small considering the mass of bloom, and even the small setting noted above is far in excess of the usual condition when there is a full crop on the remainder of the tree. It is apparent that in this position competition takes place between fruit and branch as well as between different fruits.

Evidence of further adjustment in the plum is shown by the shorter terminal growths produced during the season of heavy fruit production. LOEB (1918) studied growth adjustments in *Bryophyllum* and found that "equal masses of sister leaves produce approximately equal masses

of shoots," even when the number of shoots differs. In this form the first shoots to grow attracted "automatically the material available for shoot formation." In the plum, when there is a large setting of fruit, fruit production gains the ascendancy over vegetative growth; likewise, vegetative growth becomes uppermost in the terminal positions when the crop is limited. It appears, therefore, that within certain limits there is justification in assuming that the "struggle for existence" is a factor in the third or June drop.

Table II gives evidence of still another influence at work in the June drop. These data are taken from controlled crosses made on tubbed trees in the greenhouse where growth conditions were favorable. Pollination and aborted pistils may be eliminated, since all pollination was done by hand on normal pistils. The records as to the number set in each cross were made approximately four weeks after pollination when size differences in the ovary made it possible to distinguish between those in which fertilization had taken place and those in which it had not. The differences between the first and second column represent those pistils which fell because of lack of fertilization,—the second drop,—while the differences between the second and third columns show approximately the extent of the June drop.

In those crosses in table II on which data were taken both at the time of setting and at maturity a total of 1900 fruits which set were reduced by dropping to 726 mature fruits. In certain crosses many more fruits fell than in others; e.g., in the cross Compass \times Yellow Egg, of 1327 flowers pollinated, 652 fruits set and 8 matured. The condition in the seed of these is shown in figure E, plate 4, from which it will be noted that abscission took place near maturity. Again, in the cross Minnesota No. 12 \times Manitoba, of 180 fruits which set 80 matured. In contrast to this in the cross Compass \times Burbank, 116 fruits set and 114 matured. The general condition shown in these results is that in many of the crosses fertilization takes place but subsequently at different stages development is stopped,—a condition which shows that in some crosses fruits cannot mature for some reason, even after fertilization has taken place. The plum is comparable, therefore, to the sweet cherry (GARDNER 1913)—namely, all varieties are self-sterile, some cross-sterile and some cross-fertile.

In summarizing the relation of the pistil to sterility, it will be seen from the evidence presented that there are three distinct periods of dropping; (a) the first drop of flowers bearing aborted pistils; (b) the second drop, including all pistils in which fertilization has not occurred;

TABLE II

Showing the relationship between the number of flowers pollinated, the number set, and the number of fruits to mature in different plum crosses.

Parentage of cross		Number of flowers pollinated	Number of fruits set	Number of fruits mature
Pistillate	Pollen			
Burbank	× Minnesota No. 1 ¹	140	60	40
"	× " " "	208	39	—
"	× " " 6 ¹	379	28	22
"	× " " "	241	46	—
"	× " " 9 ¹	314	85	53
"	× " " "	274	89	—
"	× " " 12 ¹	68	5	—
"	× Surprise	457	266	121
"	× Yellow Egg	124	43	—
Compass	× Burbank	175	116	114
"	× Yellow Egg	1327	652	8
"	× Bing	34	1	1
"	× English Morello	192	0	0
"	× S. Biggareau	169	1	1
"	× Wickson	1 tree	—	275
Etopa	× P. Besseyi	1 branch	—	4
Minnesota No. 1	× Minnesota No. 6	127	2	1
" " " "	× " " "	9	0	—
" " " "	× " " 9	237	10	5
" " " "	× " " "	78	1	—
" " " "	× " " 35 ²	105	1	0
" " " "	× " " "	92	0	—
" " " "	× Terry	20	6	4
" " " "	× Yellow Egg	116	29	1
" " " "	× " "	54	7	—
" " " "	× Burbank	44	8	8
" " " "	× " "	89	18	—
" " " "	× Manitoba	169	68	61
" " " "	× Minnesota No. 9	238	48	39
" " " "	× " " "	398	96	—
" " " "	× " " 12	108	8	5
" " " "	× " " "	430	43	—
" " " "	× Surprise	291	—	96
" " " "	× Yellow Egg	101	44	17
" " " "	× Minnesota No. 6	61	3	2
" " " "	× " " 12	537	0	0
" " " "	× " " "	309	0	—
" " " "	× " " 35	209	63	50
" " " "	× Yellow Egg	65	13	13
" " " "	× Manitoba	400	180	80
" " " "	× Minnesota No. 6	207	20	3
" " " "	× " " "	341	51	—
" " " "	× " " 9	300	48	20
" " " "	× " " "	151	0	—
" " " "	× " " 35	305	84	48

¹ Burbank × Wolf.² Abundance × Wolf.

TABLE II (continued)

Showing the relationship between the number of flowers pollinated, the number set, and the number of fruits to mature in different plum crosses.

Minnesota No. 12	×	Yellow Egg	80	20	2	
"	"	35 × Minnesota No. 9	65	7	—	
"	"	" × " " 12	274	3	2	
<i>P. americana</i>	×	<i>P. americana</i>	—	—	29	
<i>P. Simoni</i>	×	Surprise	48	7	4	
Surprise	×	Burbank	108	24	0	
"	×	Double-flowering Cherry	30	0	0	
Terry	×	Burbank	11	0	0	
Yellow Egg	×	Compass	181	0	0	
"	"	×	Manitoba	42	0	0
"	"	×	Minnesota No. 6	45	1	1
"	"	×	" " 9	508	4	—
"	"	×	" " 35	49	3	—
"	"	×	S. Biggareau	28	0	0

and (c) the third or June drop, in which embryonic development is stopped. Consequently, each drop appears to be due to a different cause, and each is distinct from the others. The size differences at the time of falling are not peculiar to the plum. Preliminary observations of the apple show essentially the same condition. An outstanding feature of the evidence presented is a stimulus to growth of the pistil which results from fertilization—a stimulus which results in distinct size differences between the fruits of the second and third drops. It appears, therefore, that, excluding the loss from fungous diseases and insect injury, there is justification for placing the pistils which fail to mature in three distinct categories.

THE GENETIC PHASE OF STERILITY IN THE PLUM

Self-sterility in the plum has been investigated from three angles: (a) the elimination of gametes in the abortion of pollen and the suppression of one ovule in each ovary; (b) the genetic relationship or "affinity" between species and varieties; and (c) a study of the pollen and pistil condition as a basis for determining the type of sterility. In the treatment of each of these divisions an attempt will be made to see in how far there is justification for placing a genetic interpretation upon the condition found to exist.

The elimination of gametes

In the strict usage of the term it is not proper to refer to the elimination of gametes in speaking of pollen or pistil abortion but for convenience of presentation this may not be misleading since potentially

these would produce gametes were it not for suppression at early stages. Elimination takes place at many stages of development. In the case of pollen it was shown that, while in certain extreme hybrids there is evidence of irregularity in nuclear reorganization following the heterotypic division, for the most part pollen suppression begins at the time of microspore liberation and extends nearly to maturity. The extent of pollen suppression presented in table 4 shows that a considerable number of gametes are eliminated in this genus by means of pollen abortion.

In all plum anthers there is a considerable quantity of pollen which is never shed. Even after the pollen is shed a large number of grains are eliminated from consideration because they fail to reach the stigma. While the pollen which is lost in the process of pollination is by far the greater portion, such a loss is common to all open-pollinated plants and the element of chance deals alike with all types of pollen.

Considering now only those grains which reach the stigma, either by controlled or open pollination, sections show that while the quantity is variable, some are aborted and some are normal in appearance (plate 5, L, M). Of those which are normal in appearance some develop tubes and some do not. Aborted grains, however, never send out tubes. Again, those which germinate show great differences in the rate of tube growth, so much, indeed, that by far the larger number are eliminated so far as fertilization is concerned and are disposed of with the abscission of the style. In fact, in all the sections of pistils showing tubes in the micropyle, no cases of more than four tubes were found. It will be seen, therefore, that the number of tubes which reach the micropyle is extremely small compared with the total number of tetrads or microspores produced.

Turning now to the pistil, it has been shown that while two ovules are formed, typically one is suppressed before fertilization. This disposes of one-half the total number. In a considerable number of pistils,—those which fall at the first drop,—both ovules are suppressed. A further reduction takes place when fertilization is prevented by various causes,—this number being represented by the second drop. Consequently, as in the case of the pollen, the number of ovules which fail to function, compared with those which do function is relatively large.

Thus it will be seen that in the case of both pollen and pistil there is an extensive suppression of pollen and ovules which eliminates the gametes each would bear. The question now arises as to what causes are operating in each case to produce this condition in the germ plasm.

This phase of the subject has recently been given careful study by geneticists. They have brought forward convincing evidence which shows that there are certain factor combinations in heterozygous lines of descent which cannot undergo normal development at critical stages. The evidence upon this point has been reviewed by VALLEAU (1918) and BELLING (1918) and need not be discussed again in this connection. Suffice it to say that these researches have included both cytological studies of germ cells (TISCHLER 1908; ROSENBERG 1909; GATES 1915) and breeding tests (BRIDGES 1916; EAST 1915, and MORGAN 1914), and that the facts which have been brought out by these two methods of approach to the problem are mutually corroborative.

The suppression of one ovule in each pistil may appear at first to constitute an exception to this interpretation. And it should be emphasized that this condition does not necessarily appear to be entirely comparable genetically to that of the aborted-pollen series, since, associated with a constant suppression of one of the two ovules borne by each pistil, there may be in the same flower a complete suppression of pollen in one instance, or, in another, a normal development of all factor combinations represented in the pollen. If, however, there are certain factor combinations which cannot be brought to maturity in the $1x$ condition the suppression of one ovule would be the method of expression of this, unless certain combinations can develop in the ovule which cannot develop in the pollen. PÉCHOUTRE (1902) found that in *Prunus spinosa* two cells may start to function as embryo sacs, but that one surpasses the other in development. In the axial row of each of the two ovules in an ovary a mechanism is provided in the extra cells which do not function as embryo sacs for further elimination of genetic combinations; if the cell which would normally function as the embryo sac can be replaced by another cell of the axial row. In this way factor combinations may eliminate as many as three cells of each axial row without interfering with the typical pistil development, i.e., two ovules at the start with one suppressed generally before fertilization. This mechanism would also explain why it is possible to have, say 90 percent of the pollen aborted in forms in which all of the pistils are normal. The fact that there are a considerable number of flowers with aborted pistils, representing widely the varieties and species of this genus, suggests that factor combinations may be partly responsible for aborted pistils, especially when it is considered that the suppression of one ovule takes place before fertilization.

This interpretation of the elimination of gametes is, of course, based upon the assumption of a heterozygous condition in this genus. The

evidence in support of this has not been presented in this connection, but the great variability in fruit and tree characters shown by wild seedlings of *Prunus americana*, *P. Besseyi*, and *P. nigra* found isolated in some regions, as well as studies of a large number of F_1 seedlings of controlled interspecific crosses, show that species and varieties of *Prunus* are far from being homozygous. Furthermore, this condition would appear to be emphasized because of general self-sterility which would necessitate continual crossing.

The genetic relationship of varieties and species ✓

Considerable attention has been given to the "affinity between different varieties and species by WAUGH (1898, 1899), HEIDEMAN (1895) and others. This has been judged primarily by the ease with which crosses can be made and by the effectiveness of different varieties as pollenizers with others.

In interspecific crosses varieties of *P. triflora* have been found to cross readily with such widely separated species as *P. americana* and *P. Besseyi*. Also, *P. Besseyi* can be easily crossed with *P. triflora*, *P. americana* and *P. armenica*. A still wider cross—Compass (*P. Besseyi* × *Mineri*) × English Morello is shown in plate 4, K. Additional interspecific crosses are listed in table 11. In fact, in the fruit-breeding work at Minnesota a large number of interspecific combinations, involving two or more species, have been made, but successful crosses between any of these species and *P. domestica* are extremely rare. It appears, therefore, from these experiences, as well as from the large number of extreme interspecific crosses which have been reported in horticultural literature in the last fifty years, that in this genus the degree of sterility between species is considerably less than in some other genera which have been studied.

Something of the condition which exists between varieties is well illustrated by Whitaker, Milton, and Sophie, all of which are open-pollinated seedlings of Wild Goose. Whitaker and Milton bloom at the same time, but are inter-sterile; both, however, are fertile with Sophie, but Sophie, used as the pistil parent, is fertile with neither (WAUGH 1901). There are instances cited also in table 11 which show complete inter-sterility. For instance, Minnesota No. 9 is completely sterile when crossed with Minnesota No. 12, but fertile with Minnesota No. 35 and Minnesota No. 6. Again, Minnesota No. 12 sets fruit when crossed with Minnesota No. 35 and No. 9, but is nearly sterile with Minnesota No. 6 and Yellow Egg.

HEIDEMAN (1894) tested further the relationship between varieties by pollinating about one hundred blossoms of Wolf with pollen from six different varieties. Those blossoms pollinated with Hiawatha (*Prunus americana*) bore fruit which "were superior in size and quality to all the rest," as contrasted with those of Early Red which set no fruit. Commenting upon experiments of this nature he states (HEIDEMAN 1895) that "the union of such crosses as possess the proper degree of affinity will prove fertile, while the union of those lacking in affinity will prove sterile." He further found that "if all of the flowers of a cluster are pollinated legitimately, they will set fruit, barring accident,"—and that many plants, especially of *P. americana*, "had the power of throwing off such ovaries as were fertilized by pollen lacking in sexual affinity."

A careful study of the data in table 11, in which are shown a large number of interspecific combinations, will show in a general way the influence of the so-called "affinity" or genetic relationship upon the setting of fruit and especially upon the third drop. While no attempt has been made in this connection to determine by means of crossing the relationship of any considerable number of varieties, something of the extremes in relationship are shown by these crosses. The question which now arises is, to what extent does the failure of certain combinations to develop enter into the falling of pistils at the second and third drops. This has been discussed in part in connection with the cause of the June drop, but it has been clearly brought out in table 11 that some combinations not only set but develop fruit more readily than others. For instance, in the cross Minnesota No. 9 \times Minnesota No. 12, 537 flowers were pollinated and not a single one set fruit. This instance together with the general differences shown in many other cases between the number of flowers pollinated and those which set fruit show that there is a large mortality in all crosses.

Considering now the differences between the number of fruits which set (table 11) and of those which matured, instead of the difference between the number of flowers pollinated and of those which set fruit, it will be seen that in some of the combinations, as in the cross Compass \times Yellow Egg, a large number of fruits may set and but few ripen. This condition is further emphasized when a number of different crosses are made on different branches of the same tree, in which case it is not uncommon to get a heavy set on one branch and a light set or none on another. The same differences in setting would be expected, if instead of keeping the crosses separate by branches the same combinations were mixed on different flowers on the same branch.

One interesting feature of the crosses listed in table 11 is the difference

in time of dropping in certain combinations, the size of the fruit indicating definitely that fertilization has occurred. Generally the abscission of pistils in which fertilization has occurred takes place at about the six-week period; but in the cross Compass \times Yellow Egg, for instance, nearly the whole crop fell about one week before maturity but not until nearly the full size had been reached.

So far the discussion has dealt with arrested development during the formation of pollen or pistil, between pollination and fertilization, and after fertilization. That this is not the complete series is shown by the fact that in the seed-bed a much higher percentage of seeds of some combinations fail to grow than of others,—even when left in place for two seasons. REEVES (1917) in the Report of the Vineland Station for 1916-1917 found out that out of a total of 19,400 seeds planted only 813 germinated,—approximately 1 in 24,—and that in some varieties none grew even though as many as 2000 seeds were planted. These results agree with those at the Minnesota Fruit-breeding Farm, where, in over 150 parent combinations including many interspecific crosses, there were great differences in the number of seeds to germinate. The plum is not unusual in the small percentage of viable seeds. EAST (1915) found that germination in *Nicotiana* varies from 20 to 60 percent, and in *Oenothera*, DAVIS (1916) found a high percentage of seeds which were not viable.

Instances such as these appear to support the hypothesis that certain factor combinations are able to develop only so far,—some failing in the zygote, some in the embryo, and some not until the time of formation of the ovule or of the pollen.

In addition to the cross relationship it has been held by some investigators that there is still another influence bearing upon the sexual status in the plum, namely, a tendency toward dioeciousness. This contention has been based upon the variation in style- and filament-length found in different trees in native species. Since dioeciousness was shown to have a direct bearing upon sterility in the grape (BOOTH 1902; BEACH 1898, 1899; DORSEY 1914) and in the strawberry (VALLEAU 1918), the condition in *Prunus* reported by HEIDEMAN (1895) has been investigated further.

HEIDEMAN illustrates the following flower types in the plum: (a) the dichogamous type in which the stigma passes the receptive stage before the pollen is shed, and the reverse in which the pollen is shed before the stigma is receptive; (b) heterostyled types, those in which the pistil is

longer than the stamens, and the converse in which the stamens are longer; and (c) the bisexual group, which may be regarded as the extreme expression of the heterostyled types, in which stamens on one hand and pistils on the other are functionally suppressed. All of these types have been found in this investigation, but those of the third class are encountered so rarely that their significance from the standpoint of dioeciousness may be questioned.

In order to determine the degree of variation in the flowers of *Prunus americana*, a survey was made of the flower types borne by wild plants growing along roadsides and in gullies in the region bordering the Minnesota River west and south of Minneapolis. HEIDEMAN also made his observations of this species along the Minnesota River at New Ulm, sixty miles or so farther up-stream. The data obtained in this survey of over a hundred miles of road or river bank are presented in table 12.

TABLE 12

A summary of data obtained in a survey of the flower condition in 212 native trees of Prunus americana, showing the variability in pedicel-, stamen- and style-length.

The data on the variation in the length of stamen and style (pistil) are presented in the table in such a way as to show the relation of the length of these structures to that of the pedicel.

	Stamen- and pistil-length	Pedicel length			
		Long 14 mm ¹	Medium 10 mm	Short 6 mm	Total
Style longer than stamens	Style long (11.5 mm) ¹	2	18	3	23
	" med. (9.5 mm)	9	37	12	58
	" short (7.5 mm)	3	5	6	14
Stamens longer than style	Stamens long (11.5 mm)	7	14	2	23
	" med. (9.5 mm)	4	28	8	40
	" short (7.5 mm)	—	8	2	10
Pistil and stamens of equal length	Pistil and stamens long (11.5 mm)	1	2	—	3
	" med. (9.5 mm)	5	27	1	33
	" short (7.5 mm)	—	4	4	8
Total		31	143	38	212

¹ These figures are the mid-points of the class.

This table shows that on the basis of the relative height or length of pistil and stamens the flowers of this species can be grouped into three classes: those in which the pistil is longer and projects above the stamens, those in which the stamens are longer and project about the stigma, and those in which these two structures are equal in length. It is further shown that in each category the structures in question vary from long

to short. Pistil- and stamen-length apparently bear no constant relation to pedicel-length, since when the pistil or stamens are long the pedicel may vary from long to short.

Furthermore, the occurrence on occasional trees of partial pistil suppression, while producing functionally a staminate flower, is so variable as to have little significance in relation to evolutionary changes. In case of complete stamen suppression a functionally pistillate flower is likewise produced, but these cases are also rare.

The status of length relationship between stamen and pistil therefore appears to be that of independently varying structures. If dioeciousness is gaining expression in this species, as is contended by HEIDEMAN, flower structure has not as yet been sufficiently changed so that there are distinct pistillate and staminate flowers, although the stamen and pistil are of equal length in less than one-third of the flowers. Self-sterility, on the other hand, is prevalent in all flower types, and results in the same necessity of crossing as dioeciousness. Differences in length in a structure, at least up to a certain point, do not necessarily influence its functioning, but it is probable that in the case of the pistil, other things being equal, long styles would render fertilization more uncertain than short ones, when conditions at bloom were unfavorable.

It yet remains to be seen in how far the condition in the plum can be assigned to genetic causes and how far to nutrition. In this investigation the problem has been approached primarily from the genetic standpoint. It is probable that the two methods of approach are not as incompatible as may at first appear, and it is not the intention to minimize in this connection the bearing of those considerations broadly grouped under physiological influences.

Fruit development in the plum is apparently more dependent upon normal seed development than in some other fruits. In the apple there is considerable variation in seed development in different varieties, and the relation of seed development to size has been emphasized by WAITE (1894), KRAUS (1915), HEINICKE (1917) and others. In fact, fruit formation without seeds or even with only rudimentary carpels is not uncommon in the apple. Seedless grapes occur in a number of species but particularly in *V. vinifera*, and have long been a matter of comment by horticultural writers. A similar relation between seed formation and size prevails in the grape and the apple. The condition in the plum, however, in which typically a single seed is matured, and in which the stimulus to development must come from a single seed instead of from many,

appears to be more sharply defined. In fact, it has been shown that development may stop at any time between fertilization and maturity. This being the case the genetic relationship would be a more decisive factor than in a fruit in which there are many seeds or in which seed development is less essential to fruitfulness.

In order to get at the question at issue, suppose for the moment that fertilization has taken place in all of the pistils on a plum tree and that all set. The question is, could all pistils develop into mature fruits? If this were possible, a single spur such as that shown at N, plate 4, would have to bear approximately three quarts of fruit (assuming 18 plums per quart), or at each node, as at L and M, plate 4, six to nine plums would have to be borne. For physical reasons alone such fruit production as this could not take place on the entire tree or even on a single spur. What factors enter, then, to reduce the number of fruits?

Among the first to suggest itself is competition for available food. But it is not clear why competition alone would be the deciding factor in view of the possible adjustment as to size which takes place when the number of persistent fruits is large. This adjustment within a variety may be so great as to make a difference on the basis of size of between 18 and 48 plums per quart. Again if only one pistil in ten sets fruit (the approximate ratio in the cases shown in table 11 is one in four to set and one in ten to mature), would there be any necessity for a third drop on the basis of competition for available food? It is conceivable that competition would be considerably reduced if only one in ten were to set fruit, but from table 11 it will be seen that even this reduction in pistils does not necessarily prevent a further loss.

A study of fruit development at various stages shows some interesting adjustments in the plum. In the terminal positions on the one-year wood, as has been noted, vegetative growth is given emphasis over fruit production. On the native species—excepting *P. Besseyi*—the greater amount of fruit is produced on two-year-old wood. In either case there are instances where the terminal positions appear to favor some buds and fruiting growths over others. As a result, there is considerable variation in the time of opening of different flowers on a tree and hence in the receptiveness of different stigmas. Consequently some pistils are not only pollinated before others but in some fertilization occurs earlier than in others. Differences in time of fertilization, other things being equal is no doubt the most important single factor,—not even excepting differences in position,—in enabling one pistil to gain the ascendancy over others. The size differences in pistils are pronounced at the time of early bloom but become even more conspicuous after fertilization.

In a very detailed analysis of the factors influencing the abscission of flowers and partially developed fruits in the apple, HEINICKE (1917) shows that position, nutrition and seed development are important considerations, but these were not carefully checked by him with reference to fertilization and genetic combinations. PIETERS (1896) found that the effect of fruit-bearing upon the tissues is local, being confined in the plum and peach to "a small area in the immediate neighborhood of the fruit-stalk," but that in the apple and pear the effect is "perceptible throughout the one-year-old shoot." The local effect on the wood cylinder disappears in time. Even these adjustments, which are largely a result of differences in nutrition, do not create a condition which results in fruit setting only in certain positions, which would tend to be the case if competition were the determining factor.

Therefore, with the evidence at hand from controlled crosses in addition to that from the studies of pollen and pistil, the interpretation that normal development is determined by the factor combination either in the 1x or 2x condition appears justifiable. The influence of unfavorable factor combinations upon the June drop is especially direct in the plum, since there is but a single ovule which develops or not as the case may be. The case is different in the apple where many seeds may develop but one or more may be sufficient to furnish at least a partial stimulus to development. When there is a heavy setting of fruit resulting in increased competition for a relatively limited food supply, the uncongenial factor combinations are the first to cease development, and they are not saved either by a favorable position on the twig or by early fertilization. The difference in the time of falling at the third drop, although becoming less and less as maturity is approached, can be explained on the basis that some combinations can proceed farther in development than others. Also the greater relative number which fall at the third drop when the set is heavy would be expected, because there would be a larger number of combinations which could not develop beyond a certain point when the competition was most acute. Moreover, if this hypothesis is correct, it would appear that some combinations could be carried much farther under especially favorable conditions of nutrition than under adverse conditions. Finally, in order to check definitely the influence of position upon nutrition, careful attention would have to be given to the selection of homozygous material.

The type of sterility in the plum

The phenomena of self-sterility and cross-sterility in the plum have much in common with those reported in other forms. The outstanding

features are: (a) the constancy of expression of self-sterility even in *P. domestica* in which about one-half of the varieties are self-fertile; (b) the occurrence of cross-sterility (table 11); and (c) the slow growth of pollen tubes under the condition of self- and cross-sterility.

STOUT (1916) has divided the cases of sterility reported to date into three groups: sterility from impotence, sterility from incompatibility, and sterility from embryo abortion. These are subdivided to include variations within each group. Sterility of both the second and third types appears in the plum. The type of sterility from incompatibility is comparable to that reported in *Secale* by JOST (1907), in *Nicotiana* by EAST (1915) and by EAST and PARK (1917), in *Cichorium* by STOUT (1918), and in the apple by KNIGHT (1917). Embryo abortion, found to be so common and to contribute so largely to the June drop, has been reported in the plum by WAUGH (1899), and also in a number of other forms, such as the apple (KRAUS 1915), and *Oenothera* (DAVIS 1915 a, b, 1916).

On account of the occurrence in *Prunus* of self- and cross-sterility, of the type characterized by slow pollen-tube growth, the results of previous studies of pollen germination are of especial interest. Attempts to germinate pollen in nutrient media have shown generally that a considerable number of grains do not send out tubes (WAUGH 1900, GOFF 1901, GARDNER 1913, VALLEAU 1918, and EAST and PARK 1917). The nutrient requirements of pollen tubes are not as yet well enough known to determine whether all grains of normal appearance can be made to germinate. While germination tests may be suggestive as to the viability of pollen, they do not serve as an index to cross- or self-relationship nor can the exact line between normal and aborted pollen be drawn as yet by this method. Judging from the general appearance of plum pollen-tubes formed in artificial media and on stigmas, there appears to be no question that true tube formation occurs in artificial media even though tube growth has been short in the media used. The conspicuous knotted and twisted terminations of apparently normal tubes found in the artificial germination tests, comparable with those illustrated by GOFF (1901) throw some doubt, however, upon the pollen-tube growth being normal beyond a certain length. The point has been emphasized by STOUT (1916) and EAST and PARK (1917) that in the most carefully controlled germination tests in nutrient media, the maximum tube growth required for fertilization has not as yet been reported.

EAST and PARK (1917), in a careful review of the studies on chemotaxis conclude that there is "certainly a probability" that pollen tubes show this phenomenon, but in their experiments in which parts of the

"gynaecium" were placed in the media the results were negative although there was some evidence of increased tube growth. STOUT (1918) suggests that a "critical period in the growth of the pollen tube may result from secretions of the egg and that the different qualities of the pistil may be due to the diffusion of hormones from the gametophytes." The plum furnishes some evidence upon this point. The larger aborted pistils in the first drop become receptive even though the ovules are aborted. Pollen tubes grow in these before the flower drops. In one case, in a hand-pollinated pistil of *P. Besseyi* what appeared to be a tube was found in the carpel cavity near the aborted ovule. Tube growth has also been found in other pistils, open-pollinated, in which both ovules have aborted late before bloom. Instances like these would appear to preclude the possibility of a stimulus influencing tube growth coming from a normal egg, since in these cases development of the embryo sac had not proceeded to the formation of the egg.

The failure of so many normal-appearing grains to germinate on the same stigmas on which other grains do germinate, as is the case in the plum, raises the question as to the location of those factors which determine germination or non-germination. JOST (1907), EAST (1915), STOUT (1917), and others have emphasized the fact that the pollen tube is a $1x$ structure nourished from the $2x$ tissues of the sporophyte. This relationship is the same in self- and cross-pollination. EAST and PARK (1917) found considerable differences in tube length in *Nicotiana* and ascribed this condition to differences in the time of pollination rather than to differences in genetic constitution. In controlled crosses in *Nicotiana* (EAST 1917) 129 seeds were obtained as a result of the application of 149 pollen grains to the stigma. This is a surprisingly high proportion compared with results with the plum. A survey of the sections of a plum stigma, crossed in the greenhouse, shows that many grains were aborted and sent out no tubes, that others were normal in appearance but did not germinate, that a few sent out short tubes which at the time of fixation, 70 hours after pollination, had not extended below the stigmatic cells, and that fewer still sent out longer tubes, the longest of which extended less than one-half of the distance to the ovule.

When plum stigmas are first receptive in the greenhouse, they are often under the most favorable conditions covered with a conspicuous drop of the stigmatic fluid. When pollen is applied to such a stigma, as was the case in the above instance, all grains may be considered as entering the same substratum. Within as short a time as two to five minutes af-

ter being applied, pollen imbibes the stigmatic fluid, and even most of the aborted grains become turgid. When the amount of the stigmatic secretion is less, or when pollen is applied before receptiveness, there is greater unevenness in the matter of coming in contact with the stigmatic fluid. Such a condition would undoubtedly result in uneven germination, even if all pollen grains were viable. In the plum there appears to be another factor influencing germination and tube growth as well as time of pollination, because so many normal-appearing grains never germinate. This condition indicates that even in a fertile cross some grains are sterile, which is easily explained by the assumption that they differ in their inherited factors.

The taking up of the stigmatic fluid must certainly precede germination. In this way the protoplasmic contents of all pollen grains come in contact with the same substratum within a relatively short time; and barring selective absorption the swelling of the grains indicates that this is so. The fact that aborted grains do not send out pollen tubes shows that the growth response of the pollen comes from the nucleus instead of the cytoplasm. Up to this time, the lot of all grains would appear to be the same, and the differences noted above in germination and tube growth in the pollen of a controlled cross begin at this point. As to the style and stigma, that portion through which tubes grow would be composed of similar cells. By means of the stigmatic fluid, the gametophyte furnishes a homogeneous nutrient substance to the pollen, in the same way that the anther sap furnished a homogeneous nutrient medium to the microspores at an early stage of development. It would appear, therefore, that variations in the growth of pollen grains applied simultaneously to a receptive stigma would arise from differences in the pollen rather than in the stigma or style.

What relation, then, do self- and cross-sterility bear to the $1x$ and $2x$ conditions? STOUT (1916) points out that "a plant whose two sets of sex organs are completely incompatible is itself derived from the fusion of two cells that were compatible." This is also true of complete compatibility. This being the case, at what point in the ontogeny of a plant do the differences which bring about self-sterility arise? Cytological and genetic evidence for the most part point to a remarkable constancy in descent from cell to cell; this would be expected to continue in the stamen up to the time of the reduction divisions. The cells of the stigma and style are formed by somatic division and consequently undergo no such changes as are known to take place at the time of chromosome reduction. The δ gametes arise from cells in the anther which are in the same line of descent as those of the style and stigma, but un-

der the conditions of self-pollination they bear a changed relationship to a once congenial association, and since sterility due to incompatibility must occur at this stage, and in this relationship it appears that genetically there is lacking some factor or substance in the $1x$ condition which is present in the $2x$. Whatever the basis for this changed relationship may be, it expresses itself in self- and cross-incompatibility and prevents the union of gametes which might possibly be congenial if they were permitted to come together. It appears logical, therefore, to assume that the change comes at the time of the reduction divisions; even if the change has not as yet been defined in terms of chemistry and physiology. It yet remains to be seen whether breeding experiments show an expression of self- and cross-incompatibility sufficiently definite and constant to warrant placing this character in the same category as others which have been investigated genetically.

BAUR (1911) crossed *Antirrhinum molle*, which is self-sterile, with *A. majus*, which is self-fertile, and all the F_1 plants were self-fertile. Both self-fertile and self-sterile plants appeared in the F_2 generation. COMPTON (1912) found in *Reseda odorata* that when self-sterile plants were bred *inter se* the progeny were all self-sterile; some self-fertile plants gave only self-fertile offspring when selfed, and other self-fertile plants when selfed produced a progeny in which there were approximately three plants self-sterile to one self-fertile. COMPTON held that self-fertility in this species is a simple Mendelian dominant. In working with *Reseda odorata* he obtained results from crossing and selfing experiments which, on account of constancy of expression and the ratios obtained, can be interpreted as being in accord with the hypothesis that self-sterility is a simple Mendelian dominant to self-fertility. In *Cardamine pratensis* CORRENS (1912) found a fairly well defined relationship as to sterility or fertility when two original parent plants, B and G, were pollinated with F_1 individuals. On the basis of this relationship with both parents, he grouped the 60 plants obtained from this cross into four approximately equal classes; fertile with both B and G, 16; fertile with B but sterile with G, 16; sterile with B but fertile with G, 14; and sterile with both B and G, 14. Likewise, when the F_1 plants were crossed with the parents they fell into four classes; sterile with B, 28; fertile with B, 32; sterile with G, 30; and fertile with G, 30. CORRENS advances an explanation of his results by assuming that units, representing chemical substances—"line stuffs"—segregate in germ-cell formation. Certain discrepancies in his classes have been noted by STOUT

(1916) and by EAST and PARK (1917), which, however, do not set aside the distinct differences in cross- and self-relationship found to exist.

EAST and PARK (1917) have presented an excellent analysis of the inheritance of self-incompatibility in four species of *Nicotiana* all of which are self-sterile. The data show that as to cross-relationship the plants within either a single family or more than one family, can be grouped into classes in which each member is sterile with the others but fertile with all the individuals of every other class. In the different families these intra-sterile classes varied from one to six. These crosses show not only that self-incompatibility is inherited but that reciprocal crosses are duplicated. Both self- and cross-incompatibility were found to be due to slow tube growth. The authors propose a genetic interpretation of their results in line with recent factorial analysis. On the other hand, STOUT (1916, 1917, 1918) reports a different status of sterility in self- and cross-pollinations in *Cichorium*. His results are interpreted as showing that all grades of self- and cross-incompatibility exist in this species. In fact, self-fertility appeared in a family of red-leaved Trevis after three generations of self-sterile ancestry. The progeny of self-fertile plants do not breed true for this character. STOUT holds that "the factors which determine or prohibit successful fertilization in chicory, whatever their essential nature may be, are highly variable as to degree, specificity, and transmission in heredity." It will be interesting to note from future research in which other forms this same condition obtains.

BACKHOUSE (1912) worked with varieties of *P. domestica* and states that "there is evidence both from analogy and from the results of plum hybridization undertaken by Messrs. LAXTON BROS. in the past, to show that self-sterility is a simple unit character, self-fertility being recessive, and that the heterozygote, when self-fertilized, sets a fruit here and there, as do Mallard and River's Early Prolific.

Later, SUTTON (1918) reporting further on the work begun by BACKHOUSE, says:

"In view of the recent experiments of others two main questions arise, (1) whether self-sterility is a simple Mendelian recessive character; (2) whether the older observers were right in considering that in such cases self-steriles are fertile with the pollen of *any* other variety, or whether there are not, rather, several classes of individuals, between which there is what EAST has called 'cross-incompatibility.' As regards the first question there is nothing in our results which negatives the view that the property of self-sterility may be a recessive, but until a later generation can be tested, the only evidence bearing on this aspect of the

matter is the fact that the results with plums and cherries are consistent with the supposition that the plants consist of two larger classes, self-fertiles and self-steriles, with a smaller number of plants of intermediate properties. These and presumably some of the self-fertiles may be supposed to be heterozygous. The self-fertile class forms a fairly homogeneous group, and the occasional indications of partial self-fertility are probably attributable for the most part to errors."

Self-sterility tests indicate a different condition in the varieties of the American species of plum. The uniform occurrence of self-sterility indicates that this condition is dominant in the species tested. The limited data available show that besides the large group of self-sterile varieties (with only two possible exceptions) there are also cross-fertile and cross-sterile groups, as in *Nicotiana* and other species. So far, however, the limits of these last two groups have not been determined experimentally.

It will be seen, therefore, that while the physiology of tube growth is as yet only partly understood, much has been learned of self- and cross-incompatibility as a result of cytological and genetical studies. Inheritance studies of sterility show the behavior of this character to be quite in keeping with that of others which have been investigated genetically. There would seem to be no question concerning the segregation of a character at the time of the reduction division which produces a difference between pollen tube and stylar tissue, which, as EAST and PARK (1917) state, results in a type of sterility that is merely a physiological impediment. The type of self- and cross-sterility in the plum, therefore, is comparable with that in other forms and can be ascribed to slow tube growth.

SUMMARY

Self-sterility tests in the plum show that the varieties of the American species are self-sterile. This condition, therefore, has an important commercial bearing.

In Minnesota pollen development proceeds no farther in the fall than the archesporial-cell stage and growth in the spring begins about the first of April.

The haploid chromosome number was found to be ten in nine varieties representing seven species.

The tissues of the mature anther develop and function normally so that there appear to be no influences from this source which would contribute to pollen abortion.

The pollen mother-cell wall persists after rounding up in the mother-

cell until as late as the formation of the microspores. During the period of enlargement in the anther cavity these walls stretch and thus do not interfere with anther expansion or microspore formation.

The tetrad wall in section appears as a thin layer or membrane in close contact with the mother-cell wall and separates from it first at the angles and narrow ends of the pollen mother-cell. This wall thickens subsequent to the heterotypic division and forms the thick wall characteristic of the tetrad before the liberation of the microspores.

A thin wall is formed about the microspore before liberation from the tetrad wall and the three sutures with a single germ pore in each appear as thickening in the pollen wall begins. The mature grain is characterized by thick walls, with a furrowed surface, and fimbriated margins to the germ pore. The tube nucleus and the generative cell are much contracted in mature grains and are found near the center at the time of dehiscence.

Normal pollen development is typical of the plum and, while many aborted grains are found in all varieties under investigation and in some supposedly pure species, pollen abortion is not a cause of sterility except in rare instances where suppression is complete.

The earliest evidences of suppression were found immediately following the heterotypic division in an extreme hybrid. In other varieties suppression began after microspore liberation from the tetrad wall and grains were found in which suppression had taken place at all stages up to maturity. The percentage of aborted pollen was higher in hybrids than in species supposed to be pure.

In many of the hybrids pollen was found to break up into yellowish oily globules. This substance accounts for the fact that pollen of some varieties is "sticky" at dehiscence and is not readily blown away by wind.

Stamens may metamorphose into either petals or pistils but in the anthers affected new types of aborted pollen are not found.

In general the status of pollen development in the forms under investigation showed that neither self- nor cross-sterility could be explained upon the basis of pollen abortion.

Pistil development in the fall showed no evidence of the growing point from which the ovule is formed, but at bloom the ovary may contain two ovules in each of which there are four to eight nuclei. Typically, either before or soon after bloom one of the ovules is suppressed but many variations were found in the degree of growth before suppression.

Pistils were found to drop in three waves which are separate and distinct in point of time and size.

The first drop takes place immediately after bloom. In all flowers which drop at this time the pistils are aborted. In some abortion occurred so early that the pistils were no longer than five millimeters, in which case the pistil at bloom was always brown. In other flowers the pistils were nearly normal in size, but in these both ovules were aborted. Flowers bearing aborted pistils generally bear normal pollen.

The second drop takes place from two to four weeks after bloom and includes all pistils in which, for any reason, fertilization has not taken place. In pistils which fall at this time the conditions in the ovules are interesting. The egg remains normal in appearance for two weeks but may persist as long as 33 days after bloom. The first nuclei to break down in the absence of fertilization are the antipodals, and these are followed by the endosperm nucleus which does not divide when fertilization is prevented. The embryo sac elongates only slightly when the egg is not fertilized but the canal, into which it lengthens normally, extends full length in the nucellus until it reaches the chalaza. The ovary reaches a size of two to five millimeters in diameter before dropping.

The third or "June drop" follows the second by an interval of about two weeks. This drop is characterized by the larger size of the plums. Fertilization has taken place but embryo development has stopped. An outstanding feature of this drop is the stimulus which results from fertilization compared with the second in which this stimulus to development is lacking.

Emphasis has been placed upon the fact that pollen development in the plum is suppressed during the period of growth when the chromosome number is reduced. Abortion can be explained by the assumption that when some factor combinations are brought together complete development cannot take place during the haploid condition of the gametophytic generation. This assumption is supported by the large percentage of aborted pollen in known hybrids.

The suppression of one of the two ovules in each ovary was found to be typical. Suppression generally took place before fertilization but sometimes afterward: when it occurred before fertilization, growth was found to stop at any stage from the megaspore mother-cell to the mature embryo-sac. When one ovule was suppressed after fertilization the one in which fertilization first took place, other things being equal, appeared to gain the ascendancy.

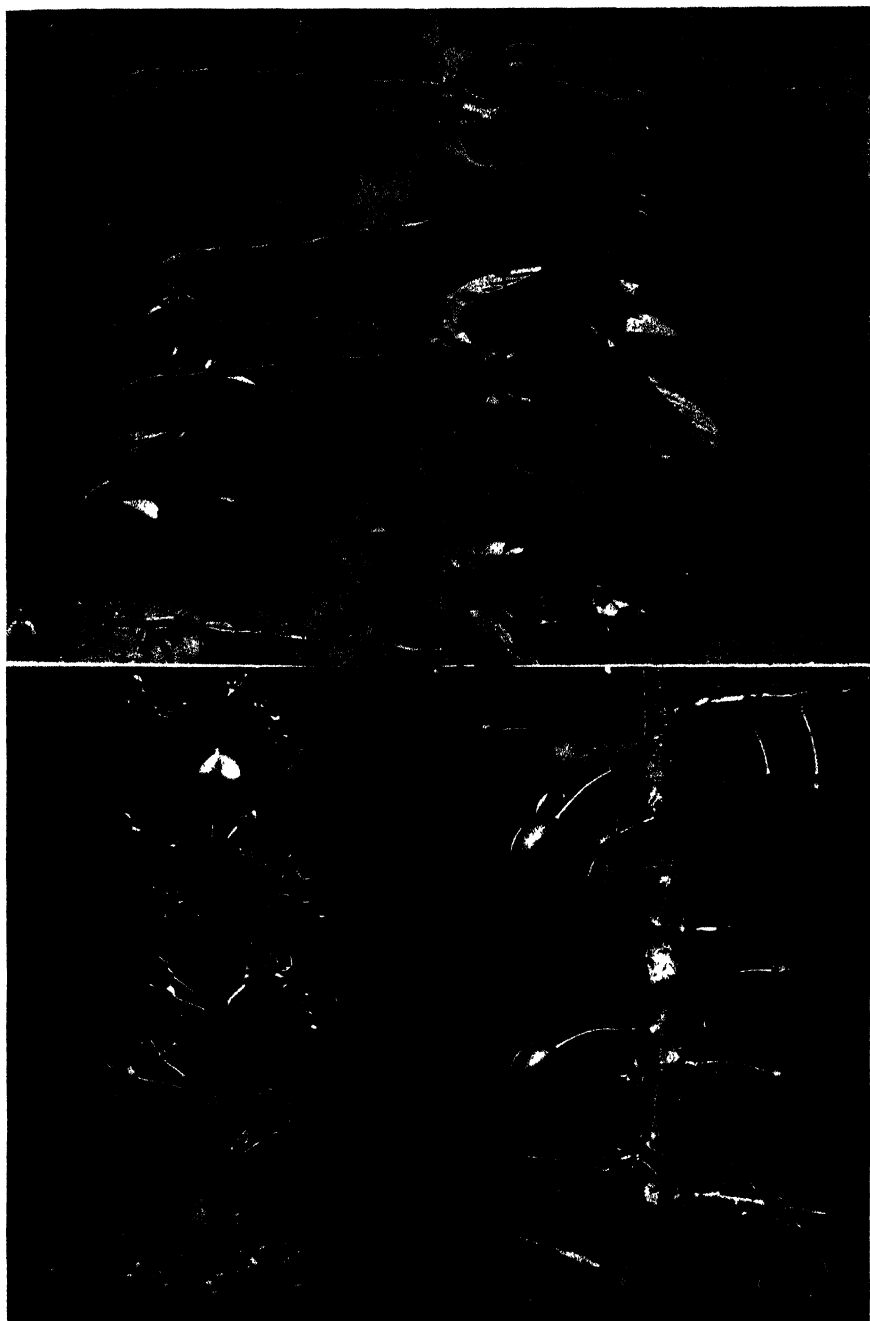
LEGEND FOR PLATE 1

A, a Sand Cherry hybrid 30 days after bloom. On the branch, a part of which is shown, there were 392 flowers. Of this number 73 had aborted pistils and only 12 set.

B, a part of a branch of *Prunus americana* 30 days after bloom, on which 138 fruits were set and only 78 pistils dropped.

C, a single twig of A (enlarged) showing the persistent calyx tubes and styles.

D, Minnesota No. 21, 31 days after bloom, showing the size differences between those setting and the pistils with unfertilized ovules which have not as yet dropped. The smaller plums illustrate the second drop. The calyx tube and style drop earlier in this variety than in that shown in C.



The reason over fifty percent of pollen may be aborted in forms in which only one-half of the ovules are suppressed can be explained on the basis that if the nucleus in the axial row of cells which would normally function as the embryo sac contains a factor combination which inhibits development its place can be taken by one of the other cells. The suppression of one-half of the ovules, however, in forms in which there is only a small percentage of aborted pollen, indicates that other than genetic causes enter into this condition in the plum.

In controlled crosses different combinations set different percentages of fruit. In some fertilization does not take place and all pistils fall in the second drop, in others fertilization occurs but a part or even all may drop. The interpretation is that different factor combinations bring this condition about through arrested development. The general relationship may be expressed as follows: All varieties under investigation were found to be self-sterile, some cross-sterile and others cross-fertile.

The type of sterility, either self- or cross-, was found to be that termed incompatibility, in which gametic fusion is prevented by slow tube growth.

Many normal-appearing grains failed to send out pollen tubes when placed on a receptive stigma under conditions similar to those in which others developed tubes. Aborted grains never send out tubes, even though they take up the stigmatic fluid.

Thus some pollen grains germinate and some do not under the conditions of the stigmatic fluid much the same as some microspores develop and some do not under the conditions of the anther sap. These differences appear to be due to something inherent in the grains rather than in their substratum, which either in the case of anther sap or stigmatic fluid, may be regarded as homogeneous throughout. This condition can be explained by differences in genetic constitution.

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LEGEND FOR PLATE 2

1.—Minnesota No. 21. Showing the origin of the tetrad wall adjacent to the tapetum. In the plum the mother-cell wall persists after rounding up in the cytoplasm, but in the grape it is dissolved in this process.

2.—*P. angustifolia*. A tetrad showing the origin of the microspore wall.

3.—Minnesota No. 21. A drawing of the germ pore and suture at the one-nucleated stage of the microspore.

4.—The vegetative nucleus and generative cell in the mature pollen grain.

5.—Minnesota No. 21. The vegetative nucleus and generative cell immediately after reorganization following division in the microspore nucleus. Compare with 6, 7, 8, 9 and 10.

6, 7.—Minnesota No. 21. The generative cell of a pollen grain which did not germinate. The stigma was self-pollinated twenty-four hours before killing for sectioning.

8.—Minnesota No. 21. A contracted generative cell in mature pollen. Note the relative size of the vegetative nucleus and the generative cell.

9.—The generative cell in the final stage. In this section the generative cell has so contracted that only the nucleus is visible and the nucleolus can seldom be made out.

10.—Same as 9, another variety.

11.—A pollen tube in the micropyle showing what is probably the generative nucleus and also the cross partition in the tube.

12.—*P. Besseyi* × *P. armenica*. A dyad with only one nucleus organized. These instances are rare. The grain adjacent showed a similar condition.

13.—*P. Besseyi* × *P. armenica*. Showing the scattered condition of the chromatin at early metaphase of the heterotypic division.

14.—*P. Besseyi* × *P. armenica*. A tetrad with an extra, small nucleus and also the heavily staining bodies in the cytoplasm.

15.—*P. Besseyi* × *P. armenica*. An intermediate stage in tetrad degeneration in which extra nuclei and rings appear in addition to the deeply staining bodies.

16.—*P. Besseyi* × *P. armenica*. A final stage in tetrad nuclear degeneration in which none of the nuclei are reorganized. The remnants of spindles indicate that division has taken place. Extreme cases like this suggest a connection between the rings in the cytoplasm and chromatin. Some of the spherical bodies can be interpreted as small nucleoli.

17.—Minnesota No. 21. The nucleus of a microspore at diakinesis showing the size at this stage compared with nuclei in the mature pollen as in 4.

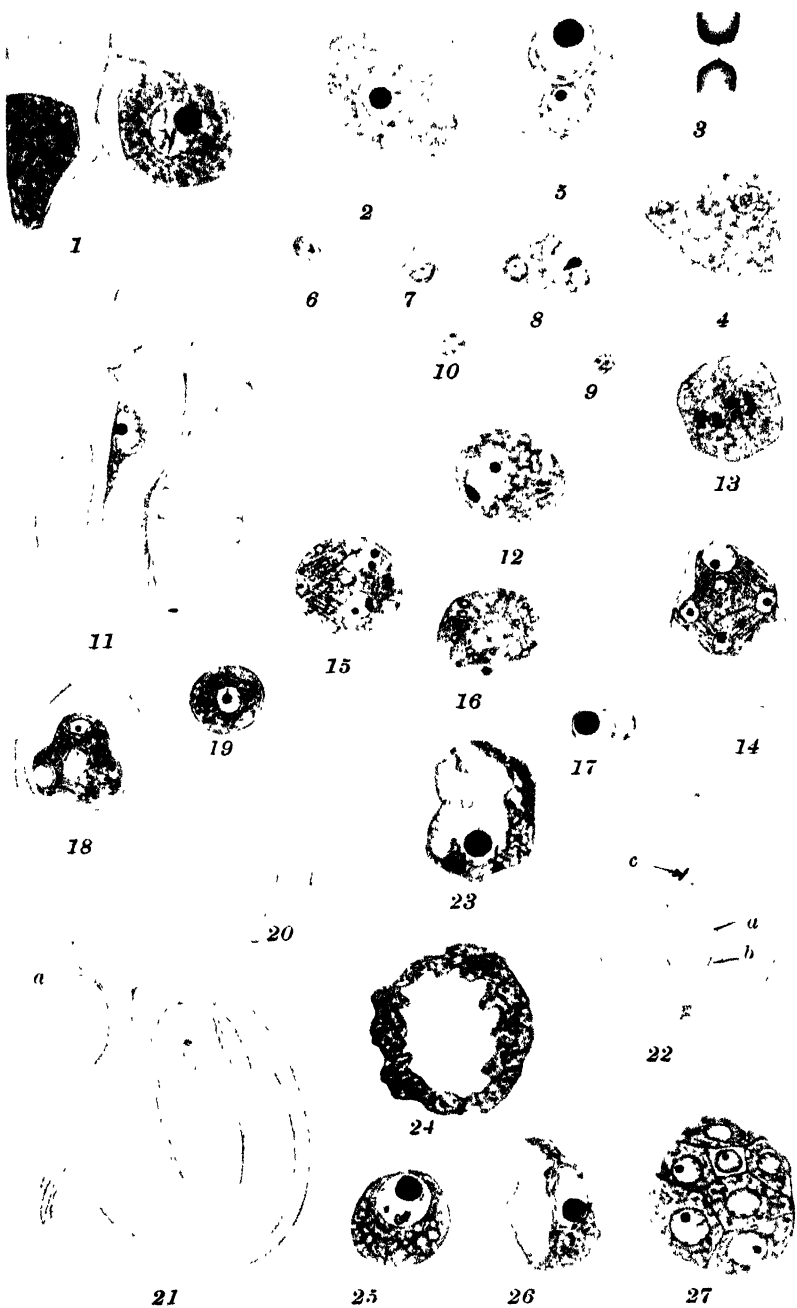
18.—*P. Besseyi* × *P. armenica*. A drawing of a tetrad showing three small nuclei in one lobe, where typically there should be only one.

19.—*P. Besseyi* × *P. armenica*. Liberated tetrads showing the extreme contrast in size soon after liberation. The smaller one has no nuclear membrane and can be interpreted as representing an early-aborted stage. The germ pore is forming in the larger one before there is much thickening in the wall.

20.—Minnesota No. 5. The winter stage of the pistil as it appeared March 22. There is as yet no growing point in the carpel cavity from which the ovules develop.

21.—Burbank. Illustrating the general morphology of the ovule 5 days before bloom.

22.—Cross section of plum showing the embryo, cotyledons, unabsorbed nucellus (a), stone tissue (b), and suppressed ovule (c).



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LEGEND FOR PLATE 2 (Continued)

23.—Minnesota No. 35. An egg 24 days after bloom. There is less disintegration than in 24.

24.—Minnesota No. 35. An egg 34 days after bloom. At this stage the canal through the nucellus extends to the chalaza but there has been only a slight elongation of the embryo sac. The egg is the only cell remaining in the embryo sac.

25.—Minnesota No. 35. The appearance of the egg 12 days after bloom, the stigma being snapped before pollination. In this case there has been no elongation of the egg sac and the cytoplasm of the egg appears vacuolated. The full number of nuclei could not be found in the embryo sac.

26.—Minnesota No. 35. Same as 25 except that the embryo sac has elongated to half the distance through the nucellus and the egg shows further evidences of disintegration on account of its vacuolization and irregularity.

27.—Minnesota No. 35. An embryo 24 days after bloom in a plum which appeared to be setting normally.

LEGEND FOR PLATE 3

Photomicrographs showing the pollen condition in some extreme plum hybrids.

A.—Wohonka \times Cherry (*P. triflora* \times *P. americana*) \times *P. cerasus*.

B.—Opata, *P. Besseyi* \times (*P. triflora* \times *P. Munsoniana*).

C.—Satsuma \times Compass, (*P. triflora* \times (*P. Besseyi* \times *P. hortulana Minor*)).

D.—*P. Besseyi* \times *P. Simoni*. Note the advanced stage of the breaking down of pollen into globules.

E.—Wolf, *P. americana mollis*. An early stage of the breaking down of pollen into globules. The walls are affected first.

F.—Opata, *P. Besseyi* \times (*P. triflora* \times *P. Munsoniana* ?).

G.—Burbank, *P. triflora*.

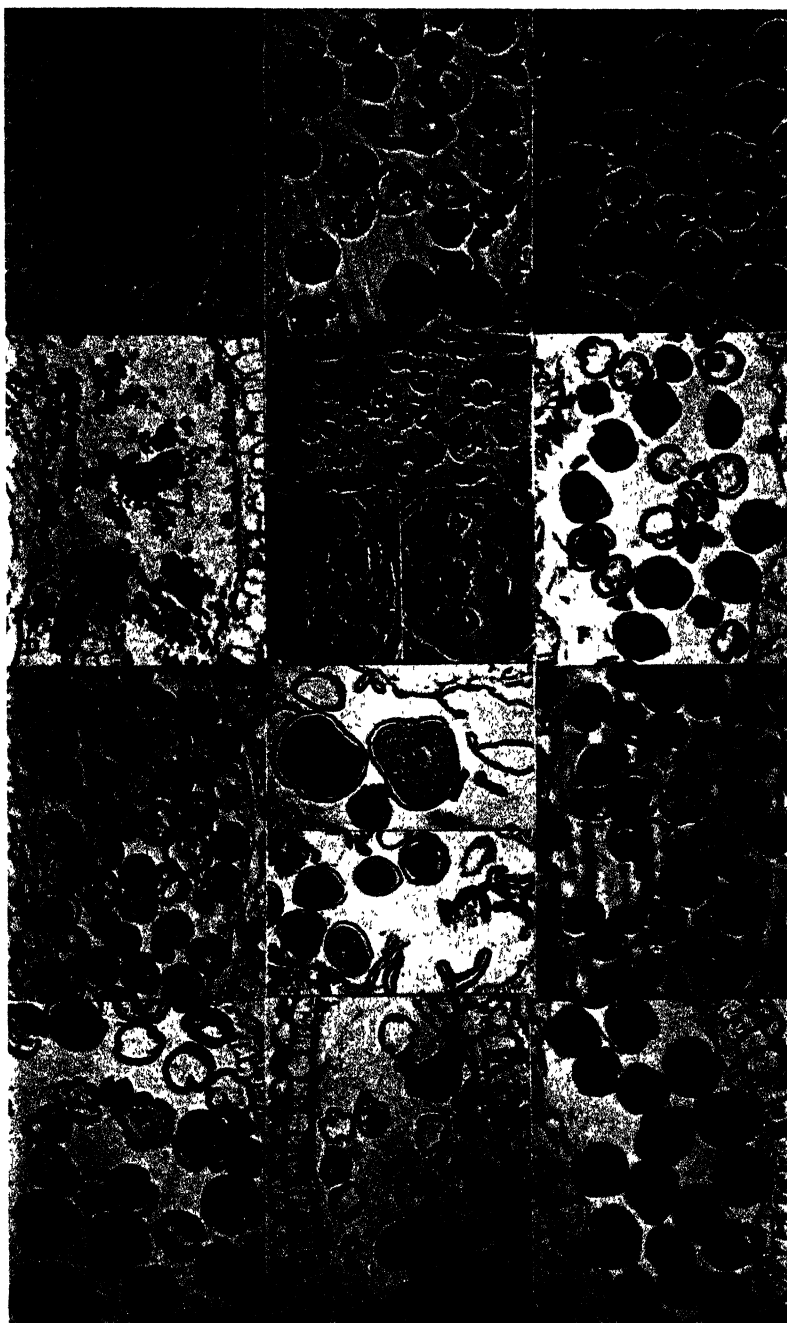
H.—*P. Pissardi*.

I.—Minnesota No. 21, (*P. triflora* \times *P. americana mollis*).

J.—Minnesota No. 17, (*P. triflora* \times *P. americana mollis*).

K.—Kamdesa.

L.—Chokecherry, *P. virginiana*. Pollen rarely aborted.



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LEGEND FOR PLATE 4

A.—An advanced stage of petalody in a *P. triflora* cross. The normal petals have been removed.

B.—Petalody in a cross between Compass and Satsuma. The normal petals have been removed.

C.—An extreme instance of pistillody in a hybrid, parents uncertain.

D.—Pistillody in a *P. triflora* cross in which only part of the stamens are affected.

E.—Embryo abortion in a cross between Compass and Yellow Egg. These plums abscised at the base of the plum and fell two weeks or so before maturity. The embryos in these cases aborted early in development but the plums reached nearly the normal size.

F.—A series showing the extremes in embryo growth in plums which were falling, in a cross between Minnesota No. 21 and Burbank, 82 days after pollination. These plums were 15 to 20 mm in diameter and the stone tissue was hard. In one seed of which sections were made the embryo was only 16 cells across.

G.—Ovule development in Stella (*P. americana* × *P. triflora*). The embryo can be found in only an occasional seed of this size but fertilization has taken place. The seed coats are brown on the three at the right. These plums were falling about one month after bloom.

H.—The relative size of the two ovules in a cross between Minnesota No. 21 and Burbank. At the extreme right both embryos are fertilized and are of nearly equal development. In each of the others one is being suppressed but not until an enlargement has taken place approximately equal to that in the unfertilized pistils.

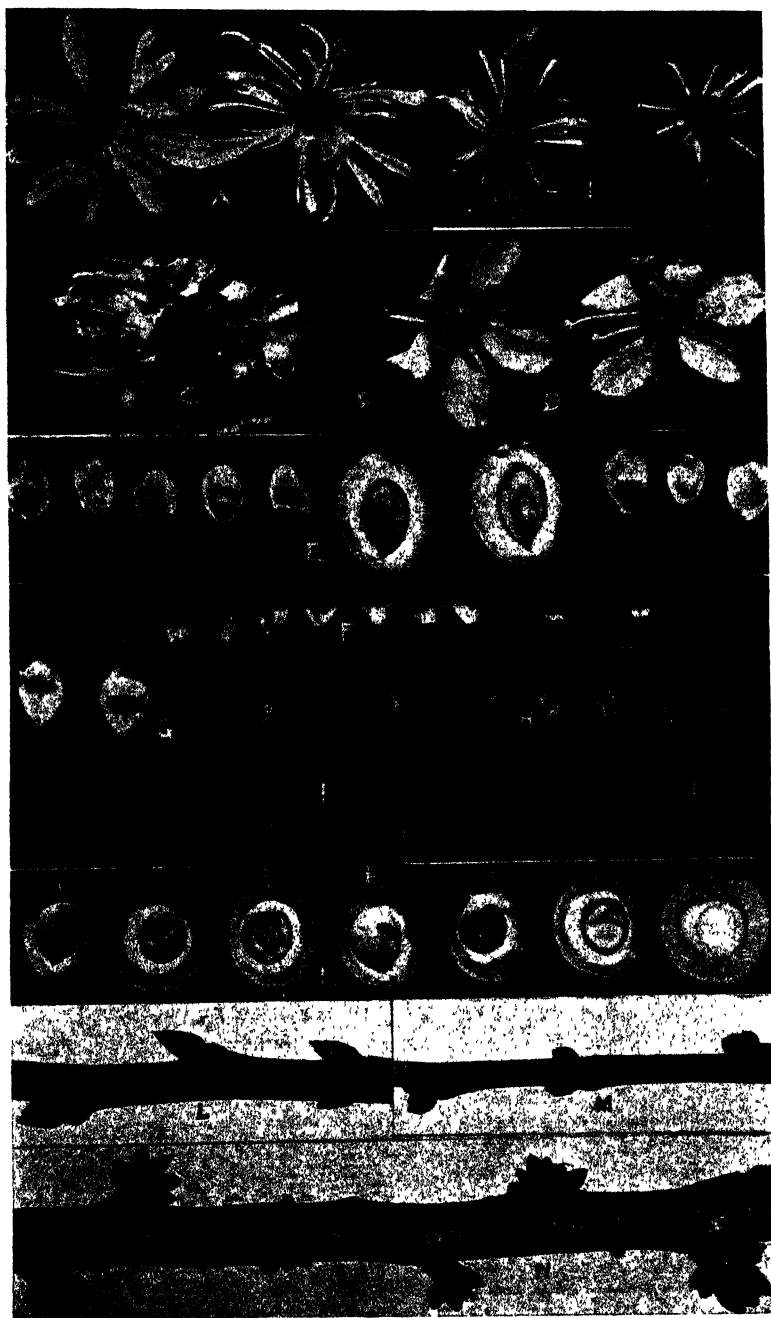
I.—The variation in the suppressed ovules in Stella compared with the normal development at the left. In this variety two seeds are often found in a pit and there is generally a partial development in the suppressed ovule.

J.—The suppressed ovules in Assiniboin (*P. nigra*). The normal seed is shown at the left. The difference between Assiniboin and Stella is largely in the time of suppression. In Assiniboin the second ovule is uniformly suppressed before bloom, so there are not normal embryo sacs to carry enlargement forward to the size of unfertilized ovules.

K.—A series in embryo development when English Morello was crossed with Compass. Those fruits with aborted seeds grew to nearly the normal size but turned yellow and fell before maturity. Fertilization had taken place.

L, M.—Fruit buds borne in one-year-old terminal twigs. These seldom set fruit in the plum. If all flowers at each node set fruit there would be 6 to 12 fruits borne at each node;—this is physically impossible on account of the weakness of the twigs if not from the standpoint of nutrition. L, Minnesota No. 21. M, Surprise.

N.—Fruiting spurs borne on the two-year wood of Burbank. There must also be a great reduction in the number of flowers borne on each spur as at each node on the one-year wood. These instances illustrate the profuse production of flowers in the plum.



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LEGEND FOR PLATE 5

A.—*P. virginiana*. Showing nearly an even development of both ovules at bloom.

B.—*P. virginiana*. The suppression of one ovule before fertilization.

C.—Cheney. The suppressed ovule 11 days after bloom. This is an extreme case of degeneration in the nucellus.

D.—Minnesota No. 12 \times Minnesota No. 21. The crowding of the suppressed ovule 17 days after pollination.

E, F.—Manitoba. The condition of the ovules in flowers with aborted pistils which fell soon after bloom.

G.—Minnesota No. 35. Showing the slight elongation of embryo sac 14 days after bloom when pollination was prevented by **snipping the stigma**.

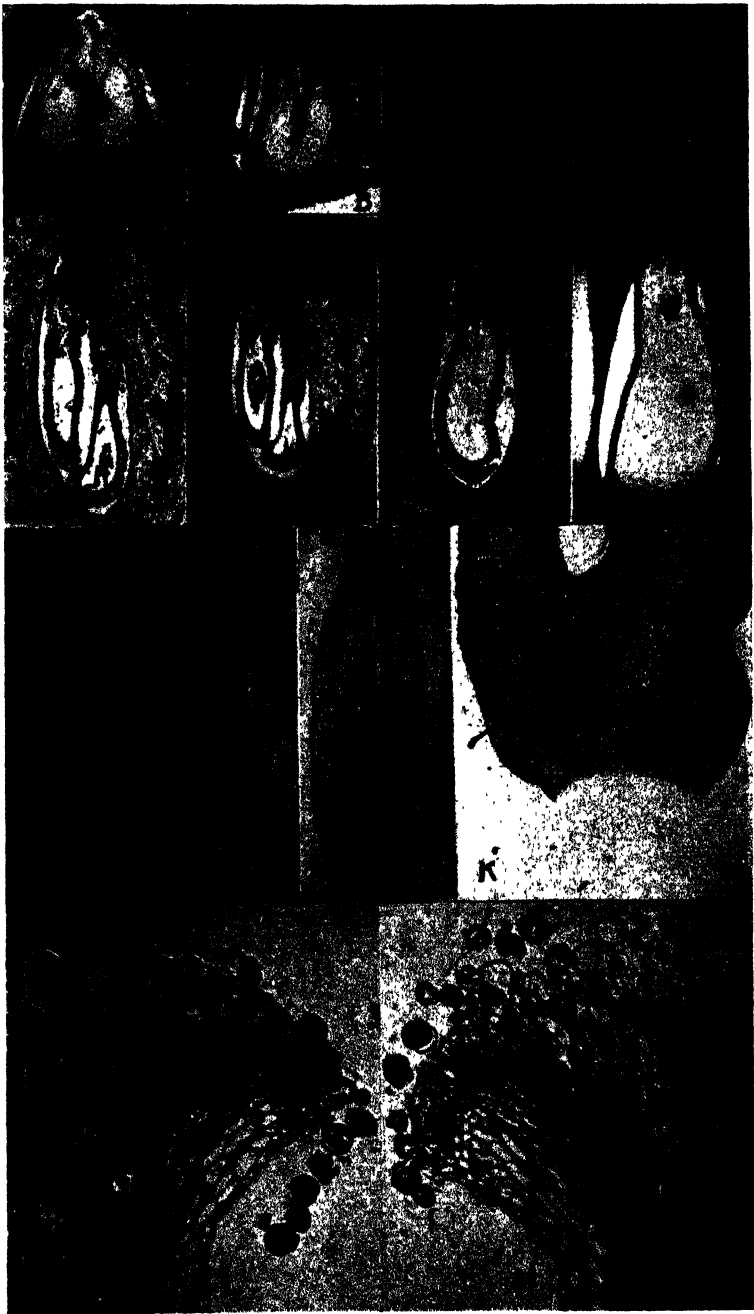
H.—Minnesota No. 35. A later stage of G, showing the extension of the embryo sac 23 days after bloom.

I.—Yellow Egg. Self-pollinated. Illustrating the embryo development two months after pollination and the three tissues in the seed, namely, a, embryo; b, endosperm; c, nucellus; and d, integument.

J.—Minnesota No. 35. The extension of the canal in a pistil 34 days after bloom in which fertilization had been prevented by **snipping the stigma**. Compare the slight embryo-sac extension with G and H.

K.—Assiniboin. Showing abscission layer at base of plum 12 days after bloom. Pistil from one-year terminal shoot. In this pistil fertilization has not taken place and the egg is degenerating. Plums of this type constitute the second drop.

L, M.—Minnesota No. 21. Pollen on a self-pollinated stigma 24 hours after pollination. Tree grown in greenhouse. Note the pollen-tube growth in self-pollinated stigma. Some normal-appearing grains have not germinated. **Aborted grains have not sent out tubes and the empty coats of grains that have germinated are still present.**



THE GENESIS OF TWINS

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In 1905 an investigation of the resemblance which exists between twins, was carried out by EDWARD L. THORNDIKE (1905) of COLUMBIA UNIVERSITY. The work was well designed to elucidate the extent and the nature of the resemblance between twins of school age, both in mind and body. Nevertheless the results differ so widely from expectation upon the current theories of heredity and the genesis of twins, that they have not been incorporated among the generally recognized facts of inheritance. It is proposed to examine the results from this point of view, and to suggest a theory of the genesis of twins which appears to reconcile the differences.

The correlations found in six mental traits range from .69 to .90 with a standard error of about $\pm .05$, while the eight physical traits range from .71 to .86 with a standard error $\pm .06$. There is thus a considerable degree of agreement between different traits, and a general level of correlation not far from .80. This is an astonishingly high value. Fraternal resemblance is usually not far from .54, so that 46 percent of the variance of the population occurs within the sibship; according to these figures, variance within the twinship must be rather less than half this quantity.

Upon the supposition that some of the twins are related fraternally and some are identical, the latter contributing little or nothing to the variance, at least half of the 50 pairs of twins covered by this investigation must be identical twins. This suggestion is supported by the distribution of the sexes, for out of 50 pairs of twins only 9 are of different sexes. Nevertheless it is quite incompatible with the actual figures, as THORNDIKE has shown under the heading "Specialization of resemblance."

It is impossible to pick out any pair of twins, much less any large group of twins, which resemble each other closely in all features. But we can go further than this. The variable used by THORNDIKE, to

measure the resemblance between any pair of twins in any factor is r where

$$r = \frac{2xy}{x^2 + y^2}$$

and x and y are the deviations of the two twins.

When x and y are the coordinates of a point on a plane, r is constant along any line passing through the origin, that is along any diameter of the frequency distribution. If θ is the angle between this diameter and either axis the distribution of the values of r may thus be used to investigate the frequency surface.

The distribution of the observed values of r is peculiar; there are concentrations at both extremes, especially near to $+1$; of 234 values derived from 39 pairs of twins for 6 physical traits, no less than 102 are above .895, and of these 27 exceed .995. THORNDIKE suggests that the form of distribution of twin resemblance is a unimodal curve comparable with that for the number of children in different families, but in this he overlooks the effects of sampling.

For a perfectly homogeneous, normally correlated population, the chance of any observation falling within the range $dx dy$ is

$$df = \frac{1}{2\pi\sigma^2\sqrt{1-\rho^2}} e^{-\frac{x^2 - 2\rho xy + y^2}{2\sigma^2(1-\rho^2)}} s^2 dx dy$$

where ρ is the correlation. If s be the distance from the centre, and θ the angle the radius makes with the axis of x , then

$$x = s \cos \theta, y = s \sin \theta$$

$$dx dy = s d\theta ds$$

and

$$df = \frac{1}{2\pi\sigma^2\sqrt{1-\rho^2}} e^{-\frac{1 - \rho \sin 2\theta}{2\sigma^2(1-\rho^2)} s^2} s ds d\theta;$$

remembering that $\sin 2\theta = r$, and $d\theta = \frac{dr}{2\sqrt{1-r^2}}$

it follows that

$$df = \frac{1}{2\pi\sigma^2\sqrt{1-\rho^2}} e^{-\frac{1-\rho r}{1-\rho^2} \cdot \frac{s^2}{2\sigma^2}} s ds \cdot \frac{dr}{2\sqrt{1-r^2}}.$$

On integrating this expression with respect to s from 0 to ∞ the frequency within the elemental sector dr is

$$\frac{1}{2\pi\sigma^2\sqrt{1-\rho^2}} \cdot \frac{\sigma^2(1-\rho^2)}{1-\rho r} \cdot \frac{dr}{2\sqrt{1-r^2}};$$

but four equal elemental sectors have the same r ; so that the frequency of a random sample falling within the range dr is

$$\frac{\sqrt{1-\rho^2}}{\pi} \cdot \frac{dr}{(1-\rho r)\sqrt{1-r^2}}$$

This expression specifies the curve of random sampling: by integrating with respect to r we obtain for the frequency of samples less than r , the expression

$$f = \frac{2}{\pi} \tan^{-1} \sqrt{\frac{1-\rho}{1+\rho}} \cdot \frac{1+r}{1-r}.$$

As soon as we examine the curve it is evident that it agrees well with the observed values of r . The ordinate becomes infinite at ± 1 , thus explaining the concentrations at the extremes, there is no mode, but the expression for the probability integral shows that the median ($f = \frac{1}{2}$), is at $r = \rho$. This is a sufficient guide in fitting the curve to the observed data. The medians for the 6 traits range from .80 to .88, and the median of the whole group is .85,

In order to test the agreement between this curve and the observed values, the sextiles are calculated from the probability integral and the range thus divided into six divisions of equal frequency; the results are shown in table 1.

P , the chance of a worse fit by random sampling, being .46, the distribution of the values of r is satisfactorily explained as being due solely to chance. It is likely, then, that the twins form a homogeneous group, with the correlations of the physical traits all not far from .85.

To make a more thorough test of homogeneity it is necessary to determine whether there is any correlation between the resemblances of the same pair of twins in different traits. For this purpose the variable r is not suitable. Its curve of distribution is far from normal and the end of its range is so cramped that 12 percent of the observations fall into one four-hundredth of the range. In addition the curve changes its form rapidly as ρ is altered, and since we are examining the possibility that different pairs of twins are samples corresponding to different values of ρ , it is essential, if the correlation is to be at all intelligible, that the

TABLE 1¹

Sextiles $\rho = .85$	Frequency	Frequency observed	Difference	d^2
— 1.000				
— .061	39	35	—4	16
+ .609	39	47	+8	64
+ .850	39	36	—3	9
+ .947	39	31	—8	64
+ .9884	39	44	+5	25
+ 1.0000	39	41	+2	4
	$\chi^2 = 4.67$		$P = .46$	Total 182

¹ In this table THORNDIKE's figures are used without correction; a more complete test, using the corrected figures divided into thirteen groups, gives an even higher value, $P = .701$.

curve, though varying in position, should be approximately constant in form.

It so happens that the distribution

$$df = \frac{\sqrt{1 - \rho^2}}{\pi} \cdot \frac{dr}{(1 - \rho r) \sqrt{1 - r^2}}$$

is capable of a transformation which precisely fulfills these conditions,

for writing $2z = \log \frac{1 + r}{1 - r}$, that is, $r = \tanh z$ and $\rho = \tanh \zeta$ we have

$$df = \frac{1}{\pi} \operatorname{sech} (z - \zeta) dz$$

a curve symmetrical about the centre, ζ , and falling off exponentially when $(z - \zeta)$ is large (figure 1).

The observed values of z may be found either from those of r , or as is desirable for high values, directly from the observations, for if x and y are the two measurements, $z = \log_e \frac{x + y}{x - y}$. There remain a few cases

in which the measurements are identical, and z nominally infinite, but a consideration of the units in which the measurements are made, and of the manner in which the probability of any assigned value of z falls off when $z - \zeta$ is large, is sufficient to restrict the value of z , with fair

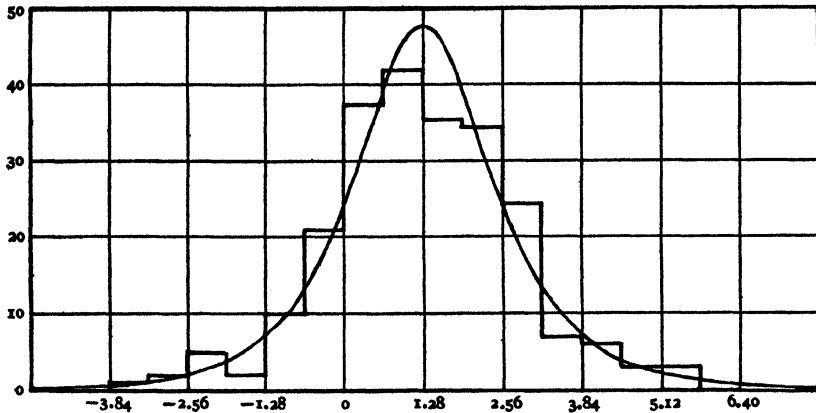


FIGURE 1

probability to sufficiently narrow limits. The figures obtained in this way are given in table 2.

When the resemblances have been expressed in terms of the new variable, a correlation table may be constructed by picking out every pair of resemblances between the same twins in different traits. The values are now centered symmetrically about a mean at 1.28, and the correlation is found to be $-.016 \pm .048$, negative but quite insignificant. The result entirely corroborates THORNDIKE's conclusions as to the specialization of resemblance; it further shows that in the population here considered, there are neither discontinuous nor continuous gradations of similarity; the population is strictly homogeneous.

The mean value 1.28, corresponds to $\rho = .856$. The transformation has not however, been made in order to determine the mean resemblance, but to test the homogeneity of the population. The value of the correlation which best fits the data may be found with less probable error, from the product-moment correlations; the mean of the values given by THORNDIKE is .79, and this value is to be preferred to the other. The differences in the mean resemblances of the six traits taken separately, are not statistically significant.

Another way of examining the distribution of resemblance is to take the means of the 6 transformed resemblances for each pair of twins. In figure 2 is shown the distribution of the 39 means, together with the curve of distribution on the supposition of uniform origin. The shape and width of this curve do not depend on the position of its centre; in the figure it is centred on the actual mean of the resemblances, 1.28.

TABLE 2

	Height	Height sitting	H/H.S.	Head circumference	Head width	Cephalic index	Total	Mean
1	2.45	4.80	1.10	2.10	1.25	3.18	14.88	2.48
2	.13	2.52	2.76	1.79	2.01	2.34	11.55	1.93
5	.64	.75	.25	2.56	1.61	.12	5.93	.99
6	— .16	1.61	2.20	2.83	.08	.47	7.03	1.17
7	.03	— .88	3.81	0	— .22	1.61	4.35	.72
8	2.07	1.82	1.45	1.39	1.19	2.66	10.58	1.76
9	.75	2.05	— 1.61	— .74	2.83	2.02	5.30	.88
10	1.82	1.65	2.57	3.04	1.05	.57	10.70	1.78
11	— .09	.89	3.93	0	2.92	3.02	10.67	1.78
12	.26	2.20	2.03	1.16	2.40	.46	8.51	1.42
13	.99	.14	.17	— .69	1.35	.44	2.40	.40
16	.43	— 1.22	— .30	2.17	1.39	3.89	6.36	1.06
17	1.47	2.83	2.06	1.85	2.30	1.79	12.30	2.05
18	1.24	1.26	0	.51	— .41	3.72	6.32	1.05
19	2.80	3.25	2.20	4.39	— .16	— 1.44	11.04	1.84
20	1.90	4.32	— .14	0	2.48	2.63	11.19	1.86
21	0	.86	0	.87	1.89	2.80	6.42	1.07
23	5.41	2.71	2.71	.41	— 2.17	— .89	8.18	1.36
24	.93	2.40	0	— 1.95	.69	2.37	4.44	.74
25	2.86	.37	— 1.95	1.03	2.71	.73	5.75	.96
26	4.10	3.64	1.61	1.36	.47	1.24	12.42	2.07
27	.36	1.85	.88	2.51	3.29	2.24	11.13	1.85
28	1.01	1.49	5.03	— 1.10	2.40	— .16	8.67	1.44
29	.35	— .26	— 3.22	— .51	.67	.93	— 2.04	— .34
30	5.14	.99	1.39	.29	1.39	.14	9.34	1.56
31	1.12	.96	2.20	1.77	3.00	1.39	10.44	1.74
32	— 1.10	2.50	1.81	2.89	2.20	.85	9.15	1.53
34	1.34	.51	— .13	.69	2.25	1.47	6.13	1.02
35	2.70	4.27	1.73	1.69	1.39	— .24	11.54	1.92
36	3.13	3.56	2.46	— 2.17	.34	— 2.67	4.65	.77
37	1.97	.62	1.25	1.10	0	.47	5.41	.90
38	1.28	.93	1.95	.34	— 2.89	.93	2.54	.42
40	— .46	— 2.30	.74	5.45	0	— 1.21	2.22	.37
41	— 1.22	— .11	.57	.92	1.95	2.23	4.34	.72
43	— .24	.37	4.50	1.61	3.45	2.94	12.63	2.10
44	.89	.14	.45	1.22	1.82	— 1.05	3.47	.58
45	2.20	.92	1.69	1.10	.59	1.10	7.60	1.27
47	1.86	2.75	0	2.20	.69	2.11	9.61	1.60
50	1.50	— .07	.46	1.10	1.10	3.18	7.27	1.21
Total	51.86	57.09	48.61	45.18	49.30	48.38	300.42	
Mean	1.33	1.46	1.25	1.16	1.26	1.24		1.284

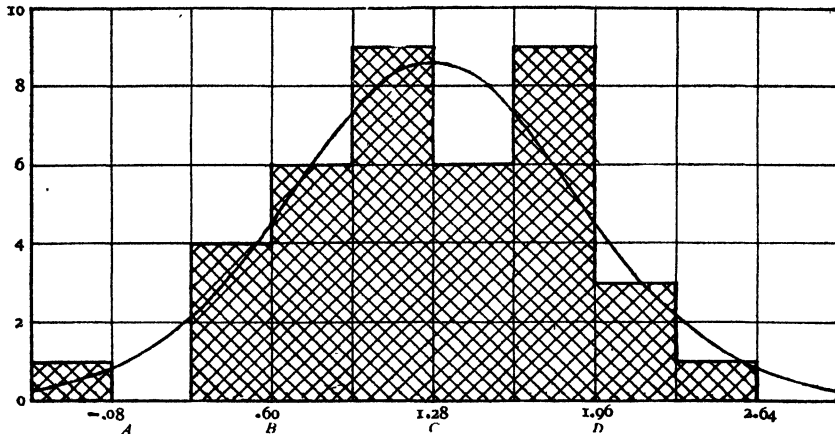


FIGURE 2

For unrelated children the curve would be centered at A ($\rho = 0$), for brothers we should expect it to be near B ($\rho = .54$), and for identical twins not far from D ($\rho = .96$); the conclusion that the origin of twins is uniform, which we have drawn from the absence of correlation between the resemblances of the same pair, is equally demonstrable from the distribution of the means. If, for instance, it were supposed that some of the twins were of fraternal origin, we should expect half of these to lie above and half below the point B. As a fact only 5 in all lie below B, so that we cannot reasonably suppose that more than 10 are of fraternal origin. Exactly the same argument shows that not more than 8 are identical twins, leaving at least 21 pairs to be accounted for by some new hypothesis. But in taking away the 9 extreme values as fraternal or identical twins, we have left the remainder with a variance considerably less than that to be expected from chance alone. There is in fact no excess at the extremes to be disposed of: the group is apparently quite homogeneous.

The same conclusion follows if we consider the 8 pairs of twins of different sexes. These must supposedly all be fraternal twins; but only one of them lies below B. The mean is at 1.04, corresponding to $\rho = .78$, slightly lower than the general average, but not unreasonably so for such a small sample, even if the different rates of development of boys and girls leaves the figures strictly comparable. The results are certainly unfavorable to the view that these twins are fraternal.

Both curves fit the data exceptionally well. For the distribution of all

resemblances (figure 1) the chance of a worse fit by random sampling is .701; for the distribution of mean resemblances (figure 2) it is .745.

The fact that the observations examined critically show themselves to be a strictly homogeneous population, with correlation much larger than that between sibs, requires a new theory of the genetic connection between twins. It is here suggested that the facts may be explained by the supposition that twins ordinarily share the hereditary nature of one gamete but not of the other.

This theory may be tested by the methods explained in a recent paper, (FISHER 1919). If the alternative types of gamete of any Mendelian character occur in the ratio $p:q$, then a pair of twins will exhibit different combinations of the three possible phases, with the frequencies shown in table 3.

TABLE 3
First Twin

Second twin	Dominant	Heterozygous	Recessive
Dominant	$p^2(p + \frac{1}{2}q)$	$\frac{1}{2} p^2 q$	
Heterozygous	$\frac{1}{2} p^2 q$	$3/2 pq(p + q)$	$\frac{1}{2} pq^2$
Recessive		$\frac{1}{2} qp^2$	$q^2(\frac{1}{2}p + q)$

If i , j , and k are the deviations from the mean corresponding to the three phases, the contribution of this phase to the product-moment of pairs of twins must therefore be

$$p^2(p + \frac{1}{2}q)i^2 + p^2 q i j + 3/2 pq(p + q)j^2 + pq^2 j k + q^2(\frac{1}{2}p + q)k^2$$

and this reduces to $\frac{3}{4}\beta^2 + \frac{1}{2}\delta^2$ where β^2 is that portion of variance due to this factor which is regularly inherited, and δ^2 is the remainder due to dominance. If mating were at random, then, the correlation between twins would be

$$\frac{3}{4} \frac{\tau^2}{\sigma^2} + \frac{1}{2} \frac{\epsilon^2}{\sigma^2},$$

where τ^2 and ϵ^2 are the totals of the elements β^2 and δ^2 contributed by the different Mendelian factors, and σ^2 is the sum of τ^2 and ϵ^2 . This may be compared to the value found for the fraternal correlation,

$$\frac{1}{2} \frac{\tau^2}{\sigma^2} + \frac{1}{4} \frac{\epsilon^2}{\sigma^2},$$

and for the paternal correlation

$$\frac{1}{2} \frac{\tau^2}{\sigma^2}$$

upon the same assumptions.

Neglecting environment, and which is far more important, assortative mating, we should have

$$t = 2f - \frac{1}{2} p$$

where t , f , and p are the correlation between twins, the fraternal and the parental correlations.

As a preliminary test we may take the figures for stature

$$f = .5433, \quad p = .5066,$$

whence

$$t = .8300,$$

a result evidently about the right magnitude.

It would not be correct to be satisfied with this verification, for the values of the fraternal and parental correlation could not be so high as they are if mating were at random. It will be found, however, that assortative mating affects these correlations nearly proportionally.

Following the methods before alluded to, it may be shown that

$$t = \frac{c_1}{+} (3 + c_2 A)$$

where c_1 and c_2 are reduction factors for environment and dominance respectively, and A the genetic association due to assortative mating.

The correlation between husband and wife is probably due to two distinct causes, the relative importance of which differs in different traits. If it were due to direct selection, which is probably the principal cause in the case of stature, we arrive at the formula

$$t = 3f - \frac{3}{2} \frac{p}{1 + \mu} - \frac{5 p^2 \mu}{(1 + \mu)^2}$$

where μ is the marital correlation. Taking $\mu = .2804$, this gives $t = .818$.

If on the other hand the marital correlation be due to indirect selection, as is apparently the case with span, we have

$$t = 3f - \frac{3}{2} p - \frac{1}{2} \mu;$$

substituting the values for span, $\mu = .1989$, $p = .4541$, $f = .5351$, we obtain

$$t = .825.$$

Evidently the agreement with the actual observations is extremely close.

The theory of the genesis of twins suggested above is in accordance with the well known fact that the father plays an important part in the causation of twinning. If the twins were formed from separate ova, fertilized by two different spermatozoa, it is difficult to see in what manner the father could influence their production. In the supposed case of identical twins formed by the division of a single zygote, the

tendency of the zygote to divide should indeed be inherited equally from either parent, but it is not easy to believe that in all the instances of paternal influence we are concerned with identical twins.

In an instance given by WAKLEY² (1895), of two twin brothers, one has a pair of twin sons, and five singly born children, the other one son; the former has through his sons three pairs of twin grandchildren, at least one pair being of opposite sex; the latter one pair of twin granddaughters. In this case a tendency to twinning, but not necessarily identical twinning, is carried by the fathers.

If we suppose that in certain cases the ovum after maturation is induced to divide into two identical portions, which are fertilized by different spermatazoa, not only is the observed resemblance of twins numerically explained, but the influence of the father is open to reasonable explanation. The division of the ovum presumably takes place during fertilization, under the direct influence of the two spermatozoa.

DAVENPORT mentions that besides the families in which the twinning tendency is carried by the males, there are other families in which it is inherited in the female line. The theory here put forward requires that the male and female gametes should be required to play different parts in the formation of twins, and the existence of two heritable qualities, the one affecting males, and the other females, is a definite confirmation of the theory.

The facts regarding the sex of twins are also in agreement with the above theory. It is generally agreed that sex in man is determined by the spermatozoon, so that the identity of the ovum does not necessitate identity of sex. The preponderance of twins of like sex, does indeed become a new problem, because it has been formerly believed to be due to the proportion of identical twins. So far as I am aware, however, no attempt has been made to show that twins are sufficiently alike to be regarded as identical really exist in sufficient numbers to explain the proportion of twins of like sex.

At least two circumstances do suggest that twins should be more often than not of like sex. For an ovum to divide and unite with two spermatozoa, instead of normally with one, it would seem to be essential that the spermatozoa should enter simultaneously and prepare themselves for union with equal speed. It may be that the necessary equipoise of attractive forces is more likely to be maintained between spermatozoa of like sex than between those of unlike sex.

In the second place much evidence has been adduced, for example by HERTWIG (1912) in the case of frogs, and by PEARL and PARSHLEY

(1913) in that of cattle, that ripeness of the ovum at fertilization is a factor in sex determination. This belief is not opposed to belief in the determination of sex by the spermatozoa, since it is possible that ova at various stages of ripeness exert selection among the gametes about to fertilize them. If, however, this belief in the effects of ripeness is well founded, it would explain why ova of the same age should tend on the whole to develop like sexes. On the other hand, if ripeness is an important factor, twins formed from different ova, and these must be upon the existing theory the majority of twins, should be generally of opposite sex, since ova simultaneously available for fertilization would ordinarily differ much in ripeness.

We may conclude then, that of the ascertainable facts concerning twins, the measurable degree of resemblance, the existence of paternal influence, the inheritance of male and female tendencies to twinning, all favor the supposition as to the origin of ordinary twins here set forth, while the distribution of sex in pairs of twins appears to present no serious difficulties.

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AN ANALYSIS OF CERTAIN CASES OF INTRA-SPECIFIC STERILITY¹

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INTRODUCTION

In the course of the experiments upon *Drosophila melanogaster* Meigen (*ampelophila* Loew) carried on at COLUMBIA UNIVERSITY, a number of mutant stocks have been obtained in which either the male or female produces few or no young. On account of both its economic and its scientific interest the general phenomenon of sterility has been the subject of investigation and discussion for a period of many years and the mass of facts which has accumulated has served to demonstrate the complexity of the problem. One of the principal conclusions from the evidence is, that the various phases of sterility are undoubtedly to be referred to diverse causes. It is a widespread belief that one of the commonest causes is the

¹ From the Zoölogical Laboratory of COLUMBIA UNIVERSITY, New York.

process of inbreeding and in several cases this idea has been supported by experimental evidence, but in the present instance, such a connection is not involved since in every race investigated the attribute in question appears in conjunction with specific somatic characters and follows the distribution of the mutant gene responsible for them, regardless of whether the stock has been inbred or not. In fact, on account of the sterility involved, out-crossing has usually been a necessity for the continuation of the strain. With the Mendelian theory as an instrument of investigation, it has been possible to demonstrate the existence of definite factors which are responsible for the degree of sterility shown. One such factor has already been indicated in *Drosophila* by the work of HYDE (1911) and two in the fowl by PEARL (1912). With the possibility of other similar discoveries in view, a brief inquiry into the nature and behavior of certain sterile stocks of *Drosophila* was begun.

The mutant races examined were seven in number and included rudimentary wings, fused wings, cleft wings, morula eyes, reduced bristle, dwarf body, and dibro. These characters are due to recessive genes widely distributed in the germ plasm, as the first three have been located in the first or X chromosome, morula and reduced bristle in the second, and dwarf in the third. Dibro has not yet been placed. In most of these strains it is the female which is markedly unproductive, but in cleft, however, the male is sterile, and since the character is sex-linked it has been impossible to obtain cleft females. In dibro both sexes have so far proved completely sterile, and a large enough number of individuals has not yet been tested to make it certain that fertile ones are not produced.

FUSED

a. *Sterility in the fused stock*

The gene representing fused has been located at 59.5 in the X chromosome. Its chief effect upon the soma is to produce a more or less complete fusion of the third and fourth wing veins and a striking degree of infertility in the female. Stock is kept up by mating the male with heterozygous females and thus obtaining normal males and normal females and fused males and fused females in practically a 1:1:1:1 ratio. Table I summarizes the results from ten such crosses. Two exceptional classes, eosin-eyed females and red males, due to non-disjunction, may be omitted and the eye colors, eosin, white and red, disregarded in the comparison of the fused and normal offspring. The total number of

TABLE I
Normal ♀ heterozygous for fused by red fused ♂.

No. of culture	Normal ♀ ♀	Fused ♀ ♀	Eosin ♀ ♀	Eosin ♂ ♂	White ♂ ♂	Eosin fused ♂ ♂	White fused ♂ ♂	Red fused ♂ ♂	Total
L	95	76	13	43	34	31	30	6	328
K	94	56	6	42	37	16	30	8	289
B	108	123	14	71	35	54	69	7	481
F	110	76	8	52	28	36	55	8	373
D	95	122		56	29	31	76		409
A	87	82		45	29	32	43		318
H	102	121		52	38	54	49		416
2	47	39	4	29	17	20	30	1	187
3	53	45	10	32	18	17	25	3	203
4	53	46	4	38	23	20	43		227
	844	786	59	460	288	311	450	33	
Total	844	786		748		761			

flies per bottle is slightly below normal but this may be accounted for in part by the fact that the temperature was below the optimum and because no effort was made to continue the counts up to the very end of the hatching period, nor were the females transferred to fresh bottles of food in order to obtain the maximum output of eggs. However, even allowing for these circumstances, we are probably justified in concluding that the heterozygous female is slightly affected in the direction of sterility by the presence in her chromosomal constitution of the factor for fused. The totals obtained for the four classes are: normal females 844, fused females 786, normal males 748, fused males 761. It is a general rule in any cross in *Drosophila* that fewer males than females are produced unless counts are continued until the bottle runs out so that the slight differences in regard to the sexes observed here may not be of particular significance. Comparing the total number of normal individuals with the total number of fused (exclusive of the two exceptional classes) we find there are 1592 to 1547,—figures which do not suggest any noticeable difficulty in the formation of the fused class from the heterozygous type. The harmfulness of the fused gene, therefore, affects the entire output of gametes in the heterozygous female rather than any one variety.

The low fertility of the fused female seems to be partly due to the fact that the normal number of eggs is not produced. Examination of the ovaries shows that fewer eggs than usual mature and by means of

egg counts it was ascertained that the number laid is below normal. Table 2 gives the data from seven crosses showing the number of eggs laid and of flies hatched therefrom. The largest number of eggs deposited by any one female was 88, none of which produced an adult fly. In the bottles where the males used were from normal stock, some of the eggs had produced larvae before they were counted and transferred

TABLE 2
Showing the number of eggs laid and of flies hatched by fused females.

No. of culture	Parents ♀ ♂	Eggs transferred	Larvae transferred	Adults produced
1	<i>fu fu</i>	23	0	0
2	" "	88	0	0
3	" "	21	1	0
4	" <i>N</i>	41	25	25
5	" "	25	4	3
6	" "	37	11	15
7	" "	37	16	5
Total		272	57	48

to fresh food. The correspondence between the number of larvae produced previous to the transfer and of flies hatched, seemed to indicate that the eggs might have been injured by handling. To test this, fused females were mated with normal males and provided with food spread in a transparent layer upon glass slides so that the eggs when laid might be easily seen and counted without necessitating their removal. Every few days the eggs were counted and the slides with eggs untouched could be placed in new bottles with plenty of fresh food. The number of adults produced was very small (table 3) even with this careful treatment—in fact the percentage of adults hatched, compared with the number of eggs laid, is lower here (5 percent) than when the first method of treatment was employed (14 percent). Data from other sources show that the percentage of eggs which hatch in normal stock may vary from 20 to 90 percent of the number of eggs laid by any female.

It was formerly supposed that fused females were absolutely sterile. Besides proving this idea incorrect, an examination of table 2 revealed the fact that the three females which were mated with fused (*fu*) males gave no flies, while all four which were crossed with normal (*N*) males were slightly fertile. This is not correlated with the number of eggs laid, for one of the crosses of fused by fused yielded the largest number

TABLE 3
*Showing the number of eggs laid and of flies
 hatched by fused females.
 Glass slide method.*

No. of culture	Parents ♀ ♂	No. of eggs laid	No. of flies hatched
B	<i>f</i> _u 6 <i>N</i>	56	1
D	" "	7	0
E	" "	35	0
G	" 4 "	23	0
H	" 6 "	21	2
M	" 3 "	12	2
Q	" "	38+	3
R	" "	39	5
Total		231	13

(88 eggs) observed from any pair in the group. The significance of the kind of male used was then investigated by making a more extended series of crosses. That it is of importance, is shown by the fact that the combination of fused with fused never produced any offspring although 49 such females were tested by being mated singly with two or more fused males, while, on the other hand, out of 62 bottles of fused females by wild (*N*) males, only 13 (or about 21 percent) were totally unproductive.

As table 4 shows, many of the 49 fertile females produced but one adult while others gave as high as 30 and 36 flies; but in the sum total of 560 offspring not one male was found.

That bar-eyed males were as effective as the normal when used as mates for fused, was proved by crossing them with twelve fused females and obtaining 263 flies. Two of the fused females, or 16 percent, were sterile. Again, all of the *F*₁ were females (table 5). Combined with the data listed in tables 2, 3 and 4, this gives 884 females and not a single male as the total number of offspring from 89 fused females, 76 percent of which (68 individuals) were fertile to at least a slight degree, provided they were mated with males from strains other than their own. As fused is recessive, the *F*₁ females were normal or bar.

The presence of a dominant factor in the hereditary complex of fused individuals does not alter the sterility of the females. Twenty-seven red-bar-eyed fused females, mated with red or eosin bar males gave no offspring although all carried the sex-linked dominant bar.

TABLE 4
Fused ♀ by wild ♂.

No. of culture	N ♀ ♀	<i>f_u</i> ♂ ♂	No. of culture	N ♀ ♀	<i>f_u</i> ♂ ♂
101	15		132	0	
102	32		133	8	
103	35		134	10	
104	30		135	1	
105	7		136	10	
106	29		137	25	
107	24		138	0	
108	2		139	10	
109	20		140	0	
110	8		141	15	
111	1		142	1	
112	2		143	3	
113	20		144	10	
114	11		145	3	
115	14		146	1	
116	5		147	5	
117	3		148	1	
118	2		149	15	
119	16		150	3	
120	1		151	36	
121	7		152	24	
122	1		153	16	
123	4		154	0	
124	1		155	21	
125	0		156	10	
126	0		157	4	
127	0		158	28	
128	0		160	0	
129	0		161	4	
130	0		183	0	
131	6		159	0	
Total			62	560	

b. Search for a factor for sterility

Although in these preliminary tests, designed to ascertain the degree of infertility of the fused stock, the results of using normal and bar males show that fused females are not to be classified as totally sterile, it is still evident that their performance is far below normal. Throughout the course of this investigation, it was constantly borne in mind that the disability here encountered might be due to the activity of a particular factor for sterility. If factors, distinct from those which

TABLE 5
Fused ♀ by bar ♂.

No. of culture	Bar ♀ ♀	Fused ♂ ♂	No. of culture	Bar ♀ ♀	Fused ♂ ♂
171	58		177	56	
172	20		178	25	
173	21		179	1	
174	10		180	2	
175	5		181	0	
176	65		182	0	
Total				263	

cause the mutations, control the productivity of the individual, we must suppose that the two kinds are closely associated in the germ cell if we wish to account for the constancy with which sterility accompanies the specific character. In other words, in order to cause such close linkage, the sterility factors must be situated in the same chromosomes as the mutant factors, probably not far removed from them. According to the theory of crossing over, it should be possible to separate the two kinds of factors if such exist, and obtain sterile races which do not show the mutation and *vice versa*. This theory is based upon the idea of the linear arrangement of factors in the chromosome threads and postulates that during some stage in the cell history, previous to maturation, when the maternal and paternal members of each pair of chromosomes twist about each other, they may permanently fuse at one or more points and when untwisting may break apart in such a manner that homologous sections of the two chromatin threads may be interchanged. If the point of fusion and breakage should occur in the interval between the loci of the factors under consideration, one of those factors would become incorporated in the opposite chromosome and the linkage between these factors would be broken. Thereafter, each one could affect the gamete to which it was distributed, free from the influence of the other.

In the absence of any definite knowledge as to the dominance or recessiveness of the sterility factor (the existence of such an entity being assumed) it becomes necessary to consider both possibilities. On the supposition that it is recessive, the fused female, with which we have been dealing, must have been homozygous for sterility, otherwise it would not have exhibited the character. A crossover which occurred in such an individual would be unavailing since, both chromosomes hav-

ing the sterility factor, this would simply be a trading of identical genes. It is in the normal female, heterozygous for fused (and for the sterility factor) that favorable conditions for effective crossing over exist. If the crossover did occur once, however, the fused female which received one chromosome free from the sterility gene, should be fertile,—at least to some extent, according to the degree of recessiveness, assignable to the sterility factor. In this case the attainment of an individual homozygous for the “not-sterile” factor would be unnecessary, for the female heterozygous for “not-sterile” should be able to found a fertile fused strain. However, the fact that the $\frac{N}{f_u}$ female does not seem to be quite

as fertile as the pure normal individual, suggests that the influence of the fused gene or of the sterility factor accompanying it, is semi-dominant, at least in this particular chromosomal complex. If this be true, a fused female heterozygous for the sterility gene might be moderately productive. If the attainment of a fused female which would have a normal fertility depends upon its homozygosity in regard to the allelomorph of the sterility factor, an egg belonging to the crossover class must be fertilized by a sperm of similar chromosomal composition before the fertile fused race can be obtained. However, it is possible that if the sterility factor is dominant, or partly so, the crossover class may already exist and the “not-sterile” factor may be passing, undetected, through the fused stock. In this case the homozygous condition might be readily obtainable.

It is of interest to note a peculiarity connected with the method of producing the homozygous crossover class when dealing with a factor like fused. Ordinarily, in such an attempt, the heterozygous female could be mated with the recessive male and their F_1 offspring, exhibiting the recessive character, could be inbred on the chance that the crossover gametes might unite in the F_2 . But where fused is concerned, out-crossing is always necessary. Fused by fused produces nothing at all. Fused females by foreign males give only females—and these are not fused. Inbreeding with the pure stock is impossible. Both kinds of individuals, both fused males and females must always be derived from the heterozygous female. In the latter type, however, the condition demanded for crossing over is always present.

Upon the conclusion of the experiments summarized in table 4, it was noted that certain of the pairs had produced quite a few individuals. As these pairs were regarded as the most likely progenitors of a fertile line,

should one be forthcoming, some of the heterozygous F_1 females from cultures 101, 103, 104, and 158 were mated with fused males and from the F_2 resulting, 63 pairs of fused brothers and sisters were made up. Here, just as in the preliminary experiment, the combination of fused with fused resulted in total sterility; showing that not one of the females had obtained by a previous crossover, either one "dose" of a dominant factor for not-sterile or two "doses" of a recessive factor for not-sterile, which would permit them to be fertile when tested by fused males. Including data given in the succeeding pages of this section, a total of 412 fused females was tested for fertility. Of these 170 were crossed with fused and the rest, 242, with wild or bar males. The highest number of offspring produced by any one individual was 86 which is far below the expectation from a fertile fly. On the assumption that the sterility factor is one unit from fused, one out of each one hundred eggs produced by a heterozygous female should belong to the crossover class of fused-fertile. If sterility is recessive, any fly formed from the crossover type of egg would indicate the fact by being fertile. If sterility is partially dominant, the homozygous recessive must be extracted before a completely fertile fused race is obtained. Fertilization of the crossover type of egg by any Y sperm gives a fused-fertile male and since there is no crossing over in this sex, the two genes remain associated during their residence in it. The union of an X sperm from this fused male with an egg of like constitution should accomplish the formation of the fertile fused female. The chances of such a meeting seem rather remote even among 412 trials, but it must be noted that should the crossover occur, the new combination of genes would remain united with as great tenacity as they formerly remained apart and, on the assumption that sterility is semi-dominant, might have travelled together for some time undetected in the stock.

c. Production of but one sex by fused females

One of the most striking points brought out by the work with fused, namely, that the females gave offspring belonging to but one sex, remained to be investigated. The suggestion that there might be some peculiarity inherent in the fused female which prevented her from producing males, made it pertinent to test the point. A solution of the problem was found by using non-disjunction.

Since the discovery of the occurrence of non-disjunction in *Drosophila*, by BRIDGES (1916), it has been known that females which possess an

extra sex chromosome, i.e., that have the unusual constitution XXY, can produce males of an exceptional kind which are formed by the union of a Y-bearing egg from the mother and an X-bearing sperm from the paternal parent, just the reverse of the ordinary process. If a fused XXY female were crossed with a normal male, we should expect the F_1 to give not only females but also the exceptional males unless the nature of the fused female prevented the formation of more than one sex. Accordingly a stock which carried the extra chromosome was so combined with the fused race that their F_2 would yield fused females, one-half of which would be expected to have three sex chromosomes, i.e., be XXY females.

One hundred and fifty-five of such individuals with eosin eyes were mated singly with red bar-eyed males. In the results from these tests the sterility was greater than in the crosses with the wild fly, as only 78 gave offspring. The expectation was that one-half or 39 of these should carry a Y chromosome and each of the 39 could throw the exceptional males. Five of these produced one to four exceptional males (table 6). These males had red and bar eyes and normal wings like the father, since their only X chromosome was obtained from the paternal parent which was red, bar and normal. This proves that it is possible for males to be produced from fused females. There were obtained also 744 bar-eyed females but no fused males, although the two classes are expected in equal numbers. Another exceptional class, that of the fused females, formed from an XX egg and a Y sperm, should also have appeared and in as large numbers as the bar-eyed males, but it was not found.

So far no fused female had ever given any offspring when crossed with males of her own kind, but in order to discover whether these XXY females might not behave differently and throw the exceptional classes, 81 fused females, half of which were expected to carry the extra Y, were mated with bar fused males (red-, white-, or eosin-eyed). No flies whatever were produced. Copulation was observed to occur in some cases and that it may be partially effective is demonstrated by the following observations.

In making counts of the eggs it was noticed that on the second day after having been deposited some of them have a small black spot at the anterior end, indicating that the chitinous larval mouth parts are formed. Removal of the chorion reveals the fact that segmentation of the body has taken place and cuticular hooks have been formed. Pressure upon the cover glass over the object may rupture the body wall and a

TABLE 6
Fused ♀♀, half of which should be XXY, by bar ♂♂.

No. of culture	Bar ♀♀	Bar ♂♂	No. of culture	Bar ♀♀	Bar ♂♂	No. of culture	Bar ♀♀	Bar ♂♂	No. of culture	Bar ♀♀	Bar ♂♂
B 1	57	4	L 6	6		S 34			S 73		
B 2	10		L 7			S 35	4		S 74	1	
B 3			L 8	8		S 36			S 75	1	
B 4	82		L 9	11		S 37			S 76	2	
B 5	6		L 10	11		S 38			S 77	1	
B 6	3		L 11			S 39	7	1	S 78	1	
B 7			S 1			S 40			S 79	7	
B 8			S 2	11		S 41			S 80	4	
B 9	24		S 3	19		S 42	6		S 81		
F 1			S 4			S 43	14		S 85		
F 2	2		S 5			S 44	4		S 86		
F 3			S 6	5		S 45			S 87	4	
F 4			S 7	4		S 46	1		S 88	3	
F 5			S 8			S 47	2		S 89		
K 1	5		S 9	1		S 48	2		C 1		
K 2	10		S 10	14		S 49	4		C 2		
K 3			S 11	9		S 50	5		C 3	2	
K 4			S 12	7		S 51			C 4	7	
K 6			S 13	4		S 52	2		C 5		
K 7	24		S 14	17		S 53	2		C 6	1	
K 8	1		S 15	36		S 54			C 7	3	
K 9	17		S 16	1		S 55	1		C 8		
J 1			S 17			S 56	1		C 9	21	
J 2			S 18			S 57	4		K 10		
J 3			S 19	3		S 58	2		K 11		
J 4	1		S 20	2		S 59	4		K 12	22	
J 5			S 21			S 60			K 13	5	
J 6			S 22	1		S 61			K 14		
J 7			S 23	19	1	S 62	3		K 15		
J 8			S 24	4		S 63			K 16		
J 9			S 25			S 64	1		K 17	36	
J 10	14		S 26	7		S 65			K 18		
J 11			S 27	32	2	S 66	4		K 19		
J 12			S 28	1		S 67			B 10		
J 13			S 29			S 68			B 11	25	
L 2			S 30	1		S 69			B 12		
L 3			S 31		1	S 70	12		B 13		
L 4	8		S 32	1		S 71	3		B 14		
L 5			S 33			S 72	1		B 15		
Total									155	744	9

tube, identified as the alimentary canal, may be squeezed out. After the second day the eggs begin to darken and show signs of disintegration.

In only two cases out of hundreds observed, have larvae hatched from the eggs and never have they been seen to pupate. The lethal influence, therefore, of the pair of fused genes may not have its full effect until the egg stages, or even the larval period, is reached.

d. *Discussion of peculiarities shown by fused*

In interpreting the foregoing results certain striking facts brought out by the experiment must be kept clearly in mind:

1. In spite of more than two hundred attempts, no cross of fused by fused has ever been successful although fused males are ordinarily fertile with females of other races and fused females may in some cases lay a good many eggs and may even produce adults when mated with males from other strains.

2. The sygotic combination of fused with fused is not fatal, as is shown by the fact that females heterozygous for fused, mated with fused males, will produce approximately as many fused males and females as they will yield normal males and females. Only a negligible difference between the two types is to be observed.

3. In the crosses of fused females with males of different kinds, only females (plus a few exceptional males) were produced, although as many as 1628 females have been obtained.

4. That the lack of males is not due to any inability of the fused females to form that particular sex, was demonstrated by using XXY females and producing, through non-disjunction, males of the exceptional class, having the characteristics of the paternal instead of the maternal parent.

An examination of the various types of crosses in which fused has been concerned, reveals the additional fact that success, or at least partial success, ensues only in those cases in which a normal allelomorph of fused is involved at some point in the scheme though not necessarily present in either of the gametes at the time when they unite to form the zygote. We are, therefore, inevitably led to the conclusion that the deleterious influence of the fused gene is of such a nature that it must—and can to some extent—be counteracted by that of its normal allelomorph in the other X chromosome, in order to insure the production of adults. Furthermore, we see that the normal X chromosome may exert its opposing influence at two points: (1) it may be effective as a member of the parental complex, i.e., in the oögonial cell before maturation, as in the instance of the heterozygous fused female where it effects the

formation of a large number (almost the normal number) of eggs, and these are capable of being fertilized by sperm even though the latter may carry the harmful fused gene; (2) when present in a gamete from a male, the normal X may have the ability to "redeem" an egg produced by a homozygous fused female and to enable it to develop normally.

One other consideration is necessary to complete an explanation for all the phenomena cited in the preceding paragraphs. This relates to the nature of the Y chromosome. The experiments indicate that it contains nothing which can counterbalance the injurious influence of the fused factor at either of the above-mentioned points. Not only does it lack this counterbalancing power itself, it cannot gain such power from association with a normal X. These considerations furnish an explanation of the facts found in a detailed analysis of the crosses made. Reference to figure 1 may make the subject clearer by giving a visual image of the types of chromosomal combinations obtained.

FORMULA 1.—*A normal female heterozygous for fused by a fused male.* All four of the expected classes, normal and fused, males and females, appear. The normal allelomorph of fused,—or of a factor for sterility lying near fused,—is carried in the normal X of the heterozygous female and is sufficient to cause the production of a large, though not quite the normal, number of eggs. It restores the eggs carrying the fused gene so that the two kinds are produced in approximately equal numbers and both are viable in combination with either the Y sperm or the sperm carrying the factor for fused in the X chromosome.

FORMULA 2.—*A fused female by a fused male.* No offspring have ever been given by this combination, which seems to be dependent on the fact that neither the eggs nor the sperm contain a normal allelomorph of fused in their chromosomal constitution. Sperm from a fused male are viable,—as witness the preceding cross; eggs from a fused female may be fertile,—note the succeeding cross; the combination of two fused gametes can also occur; the only difference to be observed between this case and the first is the absence of the normal allelomorph of fused in the maternal complex and to this circumstance must be imputed the failure to obtain any offspring.

FORMULA 3.—*A fused female by a normal male.* A single class of individuals appears,—the normal females. As we have seen, the egg production of the fused female is below normal but does not cease entirely. In type 2, the X sperm carrying fused proved unable to form a viable zygote in conjunction with an egg which had not been under

the influence of a normal X. That the X sperm from a normal or bar male possesses this ability, is demonstrated by the occasional appearance of normal-winged females in the F_1 of this cross. The non-appearance of males must be attributed to the inability of the Y sperm to serve a like function.

FORMULA 4.—*A fused female (XXY) by a bar-eyed or wild-type male.* Normal-winged females and males are given by this cross. Since maturation in this type of female differs from that of the normal, in several features, we may take up the analysis in more detail. Four kinds of eggs are expected to be formed: One with a single X chromosome which carries fused; one with two X chromosomes, each carrying fused; one possessing a fused X and a Y; one with a Y. The two kinds of sperm are the same as in the preceding cross,—one with a not-fused X, the other carrying a Y. The relationships here are similar to those in the case just discussed except for the addition of the Y in the maternal complex. As in the preceding case, only those individuals appear which are the result of a fertilization by an X sperm. This includes two types of females (XX and XXY) and the exceptional male (YX). No tests were made to distinguish whether the females actually were of different constitutions. Females containing XXX have never been found in any cross and are not expected here. The exceptional males, of which a small number were obtained, are formed by a union of an egg containing a Y with a sperm having a not-fused X. The greatest number of individuals given by any one female was 86 (table 6), which is slightly more than obtained from type 3 (tables 2, 3, 4, and 5), but by no means approaches the number produced by a female containing a normal X. This indicates that the Y chromosome cannot function like the normal X in the maternal complex in counteracting the effect of fused. It is evident that it possesses nothing which can overcome this harmful activity in the prematuration stages. The number of exceptional males expected is 4 percent of the total. Using the record of the most productive female for our computation we find that when 82 represents the number of F_1 females, the expectation for the exceptional males is 3.57. Actually 4 males appeared,—a very close approximation.

Fertilization by the Y sperm did not prove successful in a single case. The YY combination is not expected since it is supposed not to be viable, but that the X egg from a fused female is not fertilized by the Y, is explained by our assumption that the Y sperm does not con-

tain any property that can restore fused eggs nor can it acquire power so to do by association during spermatogenesis with a normal X.

FORMULA 5.—*A fused female (XXY) by a fused male.* No offspring whatever are produced. Eggs from such females and sperm from this type of male have proven viable in the preceding experiments. The particular gametic combinations expected are not impossible of realization under the proper conditions, yet no F_1 individuals are obtained. The explanation lies in the fact that the influence of the fused gene is not combated by the presence of its normal allelomorph either during oögenesis in the female or after the process of fertilization by the sperm.

RUDIMENTARY

a. The theories of prematuration and repugnance

The case of rudimentary wings, another sex-linked gene, located at 55.1 in the first chromosome, presents a less extreme example of the same sort of phenomenon as fused. The sterility of the females has been reported already by MORGAN (1912) and the theory of "prematuration" and "repugnance" suggested in connection with it. The first results indicated that the two cases possessed many similarities. MORGAN found that rudimentary flies when mated with rudimentary, were sterile; that rudimentary males were fertile with long-winged females; and that rudimentary females when fertilized by normal males gave a few offspring of both sexes. The only difference which destroys the parallel between the two cases, is in regard to the last point, for the F_1 of the fused female comprises only females while that of the rudimentary female represents males and females. It is true that most of the progeny were females, but a few males were found, in the ratio of about 1 : 300. A quotation will give MORGAN'S conception of the element of prematuration.

"In the *heterozygous* female the egg has developed up to the time of the extrusion of the polar bodies under the influence of *M* (i.e., all the normal factors are present, at least in simplex) . . . Not until the time of polar-body formation is the factor *M* lost from half of the eggs, i.e., from those eggs that may produce rudimentary offspring. Hence the relatively large number of eggs that may be fertilized by the rudimentary-winged male. On the other hand in the *rudimentary* female the egg develops without the presence of the factor *M*. If the absence of this factor, in the prematuration development, makes the egg less fertilizable by any sperm, the difference in the behavior of the two kinds of females in question can be accounted for."

PARENTS		OFFSPRING	
(1)	$\frac{+}{fu}$ (normal♀) × $\frac{fu}{Y}$ (fused♂)	<u>gives</u>	$\frac{+}{fu}$ (normal♀) $\frac{+}{Y}$ (normal♂) $\frac{fu}{fu}$ (fused♀) $\frac{fu}{Y}$ (fused♂)
(2)	$\frac{fu}{fu}$ (fused♀) × $\frac{fu}{Y}$ (fused♂)	<u>gives</u>	$\frac{fu}{fu}$ (fused♀) $\frac{fu}{Y}$ (fused♂)
(3)	$\frac{fu}{fu}$ (fused♀) × $\frac{+}{Y}$ (normal♂)	<u>gives</u>	$\frac{fu}{+}$ (normal♀) $\frac{fu}{Y}$ (fused♂)
(4)	$\frac{fu}{fu} Y$ (fused♀) × $\frac{+}{Y}$ (normal♂)	<u>gives</u>	$\left\{ \begin{array}{l} \frac{fu}{+} \text{ (normal♀) } \frac{fuY}{+} \text{ (normal♂) } \frac{fufu}{+} \text{ (dies) } \frac{Y}{+} \text{ (normal♂) } \\ \frac{fu}{Y} \text{ (fused♀) } \frac{fuY}{Y} \text{ (fused♂) } \frac{fufu}{Y} \text{ (fused♀) } \frac{Y}{Y} \text{ (dies) } \end{array} \right.$
(5)	$\frac{fu}{fu} Y$ (fused♀) × $\frac{fu}{Y}$ (fused♂)	<u>gives</u>	$\left\{ \begin{array}{l} \frac{fu}{fu} \text{ (fused♀) } \frac{fuY}{fu} \text{ (fused♂) } \frac{fufu}{fu} \text{ (dies) } \frac{Y}{fu} \text{ (fused♂) } \\ \frac{fu}{Y} \text{ (fused♀) } \frac{fuY}{Y} \text{ (fused♂) } \frac{fufu}{Y} \text{ (fused♀) } \frac{Y}{Y} \text{ (dies) } \end{array} \right.$

FIGURE I

FIGURE I.—Formulae of crosses testing fused flies. Each symbol represents a separate sex chromosome. Fused X = fu; not-fused X = +.

By the theory of "repugnance," MORGAN endeavored to explain the fact that in crosses between a female, heterozygous for rudimentary, and a normal male, the rudimentary class fell below expectation. He suggested that there was a repugnance between the rudimentary-forming gametes, that is, between the gametes which lack *M*. (In

referring to the original paper, it must be kept in mind that the terminology of the presence and absence theory was used and where the statement is made that "sperm lacking the character *M*, fertilizes with difficulty eggs lacking that same character," the Y sperm is considered to be "without" the above-mentioned *M* in the same way that the rudimentary-bearing egg is "without" it.) Statistics which prompted this explanation were given for a number of cases. In the first, there were 2061 miniature males and 479 rudimentary miniature males, a ratio of about 4.3:1 where a 1:1 ratio is expected. In the second, there were 342 miniature males to 98 rudimentary miniature, a ratio of 3.5:1. Several others gave ratios of 2:1 and 3:1.

MORGAN calls attention to the fact that his results represent an "improvement in the viability" of the rudimentary stock since its discovery. At first it had yielded 115 rudimentary males to 4773 (calculated) normal males, when equality was expected. Additional data published several years later (MORGAN and TICE 1914) showed that the disparity between the two classes of flies was due to unfavorable culture conditions rather than to a principle of repugnance. When overcrowding in the culture bottles was avoided, little repugnance was shown between any gametes forming the rudimentary classes either male or female. My observations on five matings of heterozygous females by rudimentary males, in which comparatively small numbers were involved, agreed with those of MORGAN and TICE. The total for five bottles gave 459 normal to 406 rudimentary males and 425 normal to 345 rudimentary females where equality is expected (table 7).

In the case of fused also, the principle of "repugnance" may be said to be inapplicable as is shown by the results given in table 1 from a similar cross where equality of the four classes is expected.

TABLE 7
Females heterozygous for rudimentary, by rudimentary males.

No. of culture	N ♀ ♀	r ♀ ♀	N ♂ ♂	r ♂ ♂	Total
900	124	80	130	136	470
901	102	90	108	88	388
902	96	95	119	91	401
903	69	40	56	45	210
904	34	40	46	46	166
Total	425	345	459	406	1635

In a later paper published by MORGAN (1915) the data for certain successful matings between rudimentary males and rudimentary females seemed to cast doubt upon the hypothesis of prematuration. The same type of cross in the fused stock, i.e., fused females by fused males, has never been attended with success except so far as to produce one or two larvae. That rudimentary by rudimentary may occasionally give offspring might be regarded as merely indicating that the injurious influence of the rudimentary gene is not so far-reaching as that of the gene for fused, without contradicting the general principle that particular genes may affect to a greater or lesser degree, the formation of the egg in the stages prior to the maturation divisions.

The principle of "restoration" outlined in the case of fused, is amply illustrated in the following experiment. The addition of a Y chromosome to the ordinary complex of the rudimentary female has an effect upon the formation of future classes similar to that obtained in the case of the non-disjunctional fused female, as the following data show. Sixty-five XXY rudimentary females (each X having a rudimentary gene) were mated separately to bar males (table 8). Fifteen bottles were sterile. The remaining fifty bottles gave 647 bar females, 2 rudimentary females, 41 bar males, and 52 rudimentary males. The four expected classes are represented by the four types obtained. An analysis of the cross may make this clearer. As explained in the case of the similar experiment with fused the XXY rudimentary female parent possesses three sex chromosomes, so that synapsis may occur in two ways (figure 2). There may be an XX synapsis and the Y may either pass out into the polar body or remain in the egg, thus forming eggs containing one X, or an X and a Y. In the second kind of synapsis the X is paired with the Y and the other X may pass out of, or stay in, the egg, forming gametes of the constitutions XX or simply Y as well as the XY and X classes. From analogy with other data, we assume that the XY eggs should comprise 46 percent of the total, the X eggs 46 percent, and the XX plus the Y 8 percent. All of these eggs should normally be capable of fertilization by either the X- or the Y-bearing sperm with two exceptions: the Y eggs fertilized by a Y sperm and the XX eggs by the X sperm, do not produce viable zygotes. Successful fertilizations by the Y sperm produce the recessive rudimentary classes.

In analyzing this cross, we find a second indication that the influence towards sterility of the rudimentary gene is not as great as that of fused, both because rudimentary males are obtained and because 2 rudimentary

TABLE 8
Red rudimentary XXY females by bar males.

No. of culture	Rud.	Bar ♀♀	Bar ♂♂	Rud. ♂♂	No. of culture	Rud. ♀♀	Bar ♀♀	Bar ♂♂	Rud. ♂♂
575					610		3		
576		50			611		7		
577		53	2	2	612				
578		8			613		11	1	
579		4		1	614		7		
580					615		12		1
581					616		26	2	3
582		13	1		617		3		
583					618		11		
584		16		2	619		1		
585					620		18	6	
586					621		1		
587					622		4		
588		35		3	623		5		1
589		26	3	6	624		18		1
590					625		17	1	4
591		2	1		626		4		
592					627		7		
593					628		35	2	1
594					629		6	3	1
595		13		1	630		6		
596					631		6	1	
597		9		1	632		43	9	14
598					633		6	2	1
601		1			634		9		
602		1			635		43		2
603		3		1	636		27	1	1
604					637		25		2
605		1			638	2	12	2	
606			1		639		1		
607		10			640		1		
608		3	2		641		16	1	1
609		8		2					
Total					65	2	647	41	52

exceptional females appear. The two corresponding classes have never as yet been obtained from any fused female. Here, the number of each is, however, below expectation. The rudimentary males should equal the bar females instead of being 52 to 647. This comparison, therefore, may be taken as a measure of the "restoring" power of the normal allelomorph of rudimentary. The X- and XY eggs which are produced by the rudimentary parent, all carry rudimentary and are of the same sort, no matter whether they are destined to be fertilized by an X- or a Y-bearing sperm.

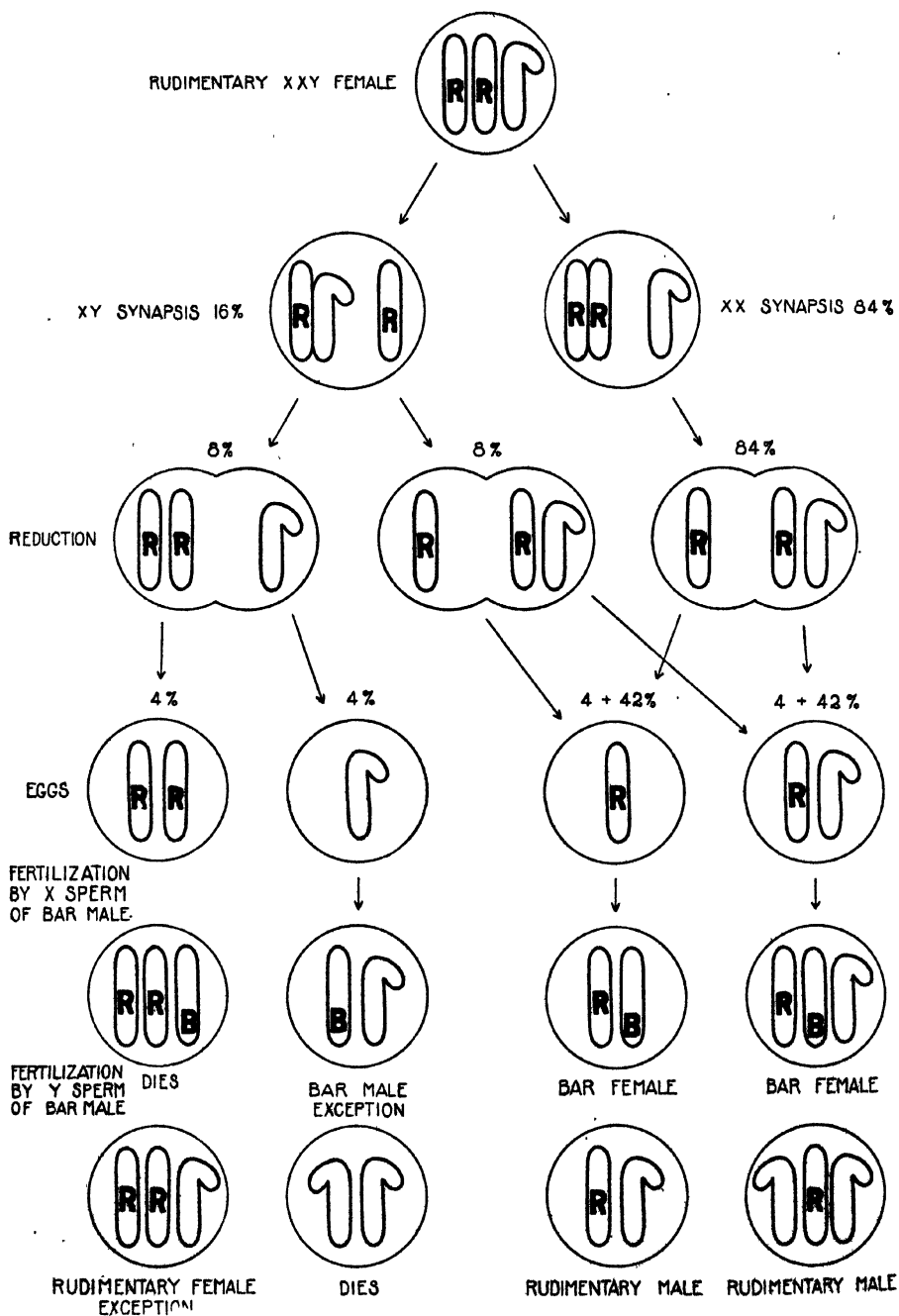


FIGURE 2.—Diagram showing the chromosomal constitution of an XXY rudimentary female, the two types of synapsis which occur in such an individual, reduction, the four possible kinds of eggs and the zygotes which would result from the fertilization

The zygotes formed from the two types of fertilization differ greatly in viability. The male zygotes are markedly fewer in number than the female. The Y chromosome which is brought in by the male-producing sperm, contains nothing, *ex hypothesi*, which can alter the viability of the egg. It does not change the percentage of sterility. The number of rudimentary males resulting from the XY and XYY zygotes is one-half the number which represents the degree of viability of the rudimentary egg when unaided by any influence from the sperm. We must suppose that a larger number of females than males is produced because the X-bearing sperm, which form the females, contain the normal allelomorph of rudimentary and have the ability in some degree to counteract the prematuration effect of the rudimentary gene upon the egg and to restore the normal process of development. The number of bar females produced compared with the number of rudimentary males, i.e., 647:52, is a measure of the "restoring" power of the normal allelomorph carried by the X sperm and operating after fertilization.

The two exceptional classes are the bar males and the rudimentary females which should be equal to each other and together should comprise 8 percent of the total progeny. The eggs which form these two classes are the result of an XY synopsis which is followed by the inclusion of the second X in the same cell with the first X, thus giving two types of eggs, —one with two sex chromosomes, XX, and one with but one sex chromosome, the Y. The first type of egg fertilized by a Y sperm, makes a rudimentary female; the second type fertilized by an X sperm, makes a bar male. This type of synopsis occurs in 16 percent of the total but as one-half of the possible zygotes derived from it are not viable, the individuals of either exceptional class are 4 percent of the total number of fertilizations by either an X sperm or a Y sperm. For example, the bar males compared to the bar females should be as 4 percent is to 92 percent, and the rudimentary females to rudimentary males should also be as 4 to 92. The total output of eggs is far below normal, probably due, as has been previously suggested, to the adverse influence upon development, of the rudimentary gene. There is no reason to suppose that the ratio of the various classes would be changed in any way from that expected in any cross involving non-disjunction. A comparison of the number of bar males to the bar females obtained, is perfectly legitimate in that the principles of both prematuration and restoration are at work in the type of union which results in the formation of both classes. A computation based on such a comparison gives 28 as the number of

bar males expected. Actually there are 41. The fact that the percentage of non-disjunction is known to be variable may account for this disparity.

In the type of reproduction which gives the rudimentary males and females, only one principle is in action—that of prematuration. As the sperm concerned in these fertilizations carries a Y as its only sex chromosome and does not possess any element to combat the injury of the prematuration influence, the principle of "restoration" is absent. Therefore, fewer rudimentary females are expected than bar males, although under normal circumstances the classes should be equal. As previously stated, the rudimentary females should be to the rudimentary males as 41 is to 92. The numbers actually obtained (2 to 52) are in quite close agreement with expectation.

b. *Search for a factor for sterility*

In continuation of the search, begun in the case of fused, for a gene governing fertility and distinct from that of the mutation involved, it was deemed advisable to test some of the individuals of the previous experiment to see whether a fertile race of rudimentary flies could be obtained. The rudimentary females from the above cross died before they were tested in regard to fertility. The other females were long-winged and had to be mated with rudimentary males in order to produce rudimentary offspring which in turn could be tested for fertility. This was done and 100 of the rudimentary females thus obtained were mated in mass cultures with their rudimentary brothers and 75 were crossed with bar males from stock (table 9). If, by means of a crossover in the heterozygous female the rudimentary gene had become separated from a sterility gene, some of these females tested might reflect this event by producing a large number of offspring. However, none of them gave as many individuals as the grandparents, one of which had yielded as many as 66 flies (table 8).

c. *Additional mutations to rudimentary*

Rudimentary has appeared twice in other stocks kept in the laboratory. One of these mutations to rudimentary, found by Mr. D. E. LANCEFIELD (1918), was tested by crossing five rudimentary females with bar males. No offspring whatever resulted, indicating that this second mutation to rudimentary was also accompanied with sterility. Whether a previous mutation to sterility had occurred at a point adjacent to the rudimentary locus, and was made apparent only by the modifying action of the rudimentary gene is a question not settled by the evidence.

TABLE 9
Rudimentary ♀♀ by rudimentary or bar ♂♂.

No. of culture	Parents		F ₁ ♀♀	F ₁ ♂♂	Total
	♀♀	♂♂			
702	11	× 6 rud.	1	2	3
703	6	3 "	3	1	4
704	10	5 "			
705	4	12 "	3	10	13
706	3	4 "			
708	11	6 " +1B'	10	8	18
709	4	2 "	3	8	11
710	3	3 "			
711	9	7 "	18	±5	±23
713	10	10 "	6	7	13
719	12	7 "	2	5	7
714	10	10 "	6	5	11
720	7	7 "	2		2
715	33	13 B'	20	3	23
716	18	7 "	28	7	35
717	22	33 "	13	8	21
718	2	2 "			
Total	175		115	69	184

Dr. A. H. STURTEVANT found rudimentary a third time; in this instance, in a strain of black purple curved flies. A cross between this newest rudimentary and a normal female heterozygous for the original rudimentary gene gave normal and also rudimentary males and females. Had the new mutation been dependent upon a gene different from the first, their F₁ should give only normal offspring, as each parent would carry in its chromosomal constitution the normal allelomorph for the opposite factor. Since the recessive class of rudimentary females appeared in the F₁ we must conclude that each parent contributed the same gene and that the third mutation of rudimentary was therefore identical with the first.

The STURTEVANT stock was then tested for the possible occurrence of a fertile individual. Of 72 females, crossed with bar males, none yielded more than 57 offspring and 45 (about 62 percent) did not reproduce at all (table 10). The sterility here seems to be more pronounced than in the first rudimentary stock but this is perhaps to be referred to temperature conditions rather than any real difference in the nature of the gene. This strain was kept up by mating some of the heterozygous females with rudimentary males and the tests were continued upon their offspring. Twenty-seven of the rudimentary daughters were mated with one or

TABLE 10
Rudimentary ♀♀ by bar ♂♂ (STURTEVANT stock).

No. of culture	Bar ♀♀	Rud. ♂♂	Total	No. of culture	Bar ♀♀	Rud. ♂♂	Total
1001				1037			
1002				1038			
1003				1039		1	1
1004				1040			
1005				1041			
1006	1		1	1042	11	2	13
1007				1043	2		2
1008				1053	5		5
1009	1		1	1054	+		+
1010				2002			
1011				2001	+		+
1012	1		1	2003			
1013	5		5	2004			
1014	2		2	2005	1		1
1015				2006	3		3
1016				2007			
1017				2008	1		1
1018				2009			
1019				2010	1		1
1020	1		1	2011			
1021	2		2	2012			
1022				2013			
1023				2014	2		2
1024				2015			
1025				2016			
1026	1		1	2017			
1027				2018			
1028				2019	1		1
1029				2022			
1030				2023	2		2
1031				2024			
1032				2025	2		2
1033				2026			
1034		2	2	2028	+		+
1035	2		2	3221	31	26	57
1036				3225	+		+

more rudimentary brothers (table 11) and 14 of these bottles gave offspring. Nine rudimentary females, out-crossed with bar males, all reproduced, but none of either of these groups gave more than a dozen flies (culture No. 4095 to 4103, inclusive). In addition 94 rudimentary females were crossed with bar males (Nos. 4020 to 4105 and 4152 to 4160). An exact record of their performance was not kept but a large number of offspring was not given by any one of them. Among the 202 females of the STURTEVANT strain, tested for sterility, a completely fertile individual was not found.

TABLE II
Rudimentary ♀♀ by rudimentary ♂♂ (STURTEVANT stock).

No. of culture	♂ and ♀ offspring	No. of culture	♂ and ♀ offspring	No. of culture	♂ and ♀ offspring
3251	2	3240	3	3248	1
3252	1	3243	2	3249	1
3236	2	3245	2+	3250	4
3237	2	3246	3	3262	1
3239	3	3247	8		

Thirteen other cultures gave no offspring.

MORULA

"Morula" eye is dependent upon a gene located at 106.3 in the second chromosome. It is characterized by great irregularity in size and protuberance of the ommatidia. In addition, the reduction of the bristles on the thorax is of such regular occurrence that their condition may be taken as diagnostic of morula when the eye disturbances are scarcely detectable.

The females lay only a few eggs. Five, which were observed for ten days, gave respectively 0, 0, 4, 9, and 4 eggs, none of which hatched. The males are probably perfectly fertile as no difficulty is experienced in keeping up stock by means of crossing them with the heterozygous female.

As the female shows a very high degree of sterility, it is evident that the few eggs which are laid are seldom capable of normal development. Three hundred morula females were mated with morula males in small mass cultures. No offspring were obtained. In order to discover whether males, other than her own kind, were more effective as mates, which had been observed to be the case with fused, 107 bottles were made up, each containing one female and 1 to 3 red bar or eosin crimson males. With sterile stocks difficulty is often experienced with the growth of mold upon the banana used as food, which may prevent the development of flies that might come through if it were not for this adverse condition. In this experiment the expedient was tried of inoculating the 107 bottles with the eggs of yellow flies so that the larvae of the more viable stock might churn up the food and by their activity destroy the mold. The adults of this stock could easily be distinguished from any gray morula flies which might hatch at the same time. Even with this precaution, the percentage of fertility was not materially increased

as only one of the 107 females gave offspring. She produced but two individuals, both normal-eyed females. Crossing these heterozygous females with morula males gave normal and morula males and females. Some of the males had yellow bodies, showing that the original morula grandparent had been fertilized by one of the yellow flies with which the bottle had been inoculated instead of by a red bar or eosin crimson male. Probably, no particular significance attaches to this fact. Eighty-eight of the F_2 morula females were mated with morula brothers, either yellow or gray-bodied, but none of them reproduced.

These figures indicate the very high rate of sterility among morula females as only one individual in the 500 tested gave offspring. The same considerations in regard to the possibility of sterility being dependent upon a separate gene, apply here as in the case of fused or rudimentary. Each one of these females was derived from a heterozygous parent which fulfilled the conditions necessary for crossing over. Had a chromosome containing morula, but free from the factor for sterility, been made up during this process, the occurrence would have been detected in the individual formed from it, provided sterility is recessive. Even if it is semi-dominant, either by itself or as a modifier of morula, we might hope that the homozygous recessive, i.e., for fertility, might have been formed during so many tests. That no morula female evinced normal fertility must be taken to indicate that sterility is dependent upon a gene lying exceedingly close to morula or that it is one of the manifold effects of morula itself.

REDUCED BRISTLE

Among the mutant stocks which proved sterile was one named "reduced bristle" because of the fact that the bristles on the thorax are reduced either in size or number. Stock is kept up by mating the heterozygous female with reduced-bristle males as the reduced-bristle females showed a high degree of unproductivity.

Observations on the egg-laying capacity were made in but two cases. The first female laid no eggs at all during a period of 9 days and the second laid 77 in 11 days. None of these hatched.

Of 70 reduced-bristle females tested for fertility with Australian (wild type), bar, California (wild-type) or eosin crimson males, only three gave offspring, producing 1, 37 and 16 flies, respectively. The difficulty of obtaining males which was evident in the fused and rudimentary stocks was conspicuously absent here as No. 53 gave almost half again as many males as females, i.e., 23 to 14, and No. 54, 7 males to 9 females.

This fact is in no way a contradiction of our theory of "prematuration and restoration," but is rather a confirmation of it and is explicable on the ground that here we are not dealing with a sex-linked factor.

The homozygous reduced-bristle females, as we have seen, lay very few eggs. We assume this is owing to the fact that the harmful influence of the pair of reduced-bristle genes (or of the sterility genes lying adjacent to them) is unchecked by any normal allelomorph in the prematuration stages of oögenesis. Some of these eggs, however, may be fertilized and brought to complete development by the redeeming influence of the gamete from the male. Since reduced bristle is not sex-linked but lies in the second chromosome its normal allelomorph is contained in duplex in all males used in the experiment. Therefore, all sperm, whether X- or Y-bearing, also carry an autosome which possesses an allelomorph to reduced bristle (or sterility) and have the power to counteract, in some measure, the injurious reactions started in the egg by reduced bristle, and produce viable zygotes. Since both male- and female-forming sperm have this capacity, both sexes are represented in the F_1 . As reduced bristle is recessive, the F_1 flies appear normal.

The three productive lines were continued by out-crossing some of the bar-eyed daughters heterozygous for reduced bristle, to reduced-bristle males from stock and the reduced-bristle F_2 females obtained in this way were tested to see whether they were fertile. Among the F_2 , of line 53, 16 females were mated in small mass cultures with males of their own kind but produced no flies. Twelve females from line 54 and 25 from line 52 gave similar results, showing that in a total of 125 females, not one possessed the reduced-bristle factor free from a factor for sterility, although three of them were not totally sterile.

DIBRO

The name "dibro" is an abbreviation of the three principal characteristics: *dichaete*, *beaded* and *rough*, exhibited by one of the strains which proved sterile. Besides having reduced bristles, beaded wings, and irregular eye facets, the abdomen is noticeably shorter and smaller in circumference than in the normal fly. The stock died out before the gene was located in any chromosome. BRIDGES, who found this mutation, reports the appearance of only one male, which was sterile, and in my cultures the four males which appeared were so weak that they lived only a short time.

The ovaries of the dibro female are small and the eggs remain im-

mature. Observation of twelve females, mated with bar or dibro males, for periods of from 9 to 16 days showed that no eggs had been laid.

Three females mated with bar males produced no offspring during the five or six days which they lived. Five more, mated with bar males, also showed sterility. The mutation appeared in eosin-eyed cultures and stock is maintained by making a large number of pair matings from the bottle which threw dibro. The appearance of dibro from any one pair shows that each one of the parents must have carried the gene in question and indicates the proper line from which to continue the stock.

DWARF

Aside from the fact that they are often lighter in color than the normal stock, dwarf flies show few differences from the wild type except that implied by their name. Frequently they are smaller than the wild fly, being approximately one-half the normal size but the range of variability is very great and some individuals may overlap the normal. The eggs also are smaller than those of the wild fly. The long axis is considerably shorter giving the egg a chubby appearance. Not all the females lay eggs. Of the eight females mated with either bar, dwarf, or wild males, and placed under observation for various periods of time, three produced no eggs, the other five gave 143, 50, 143, and 108, respectively (table 12). In the batch of 143 eggs, 54 of them dried up and therefore must not be included in the fertility percentage. From the total of 291 eggs, all from crosses in which bar or normal males had been used, only seven flies hatched. They were either bar or normal females. It is to be noted that no males appeared, but this fact is probably not to be regarded as significant in view of the small numbers involved.

TABLE 12

Showing number of eggs laid by dwarf females and of flies hatched therefrom.

No. of culture	Parents		No. of days observed	Eggs laid	Flies produced
	♀	♂			
1	<i>d_w</i>	<i>B'</i>	6	0	0
2	"	<i>d_w</i>	12	0	0
3	"	<i>N</i>	12	89+54	4
4	"	<i>B'</i>	14	50	1
5	"	"	9	43	0
6	"	"	14	1	0
7	"	"	14	108	2
8	"	"	11	0	0
Total				345	7

In a previous experiment, in which no counts of the eggs were made, the fertility of 31 adults was tested and found to be zero. Two of the heterozygous females obtained from the group of 108 eggs in the egg-count experiment were mated to dwarf males and gave 160 normal flies to 21 dwarf females and 25 dwarf males. Twenty maroon dwarf females from this cross and 4 from another F_2 group, were placed with bar or dwarf males, but did not yield a single individual. To sum up, 3 out of 63 females proved slightly fertile.

CLEFT

Cleft (denoting a peculiarity in venation) is a sex-linked recessive (locus 65). Only males of this mutant type have appeared. Since they are sterile, it has not been possible as yet to obtain any cleft females. Stock is kept up by mating the daughters of any female which has thrown cleft.

Examination shows that the testis is usually misshapen, often with the parts more or less fused, but containing live sperm. Copulation has not been seen to take place. Seventy-four males were put in separate bottles to which were added females from California (wild type), Australia (wild-type), bar or eosin crimson strains and, in two cases, normal females heterozygous for morula. These bottles had previously been inseminated with larvae from yellow stock in order to keep the food in good condition. Four of them gave a few offspring but until tested it was not certain whether their paternal parent had been a cleft male or a yellow male that had chanced to hatch early. When the female offspring mated, the kind of males they produced would indicate their ancestry, for both cleft and yellow are sex-linked and would therefore appear in the F_1 . The results of the test of the daughters gave yellow males in every case, but no cleft, proving the paternal parent to have been yellow.

In regard to the possibility of separating an (assumed) sterility factor from that of cleft it is to be noted that the separation could not take place in the male, since crossing over does not occur in the male of *Drosophila*. In the heterozygous female from which cleft males were derived, however, fertile sons might be produced. In the seventy-four tests made such an individual was not discovered.

GENERAL DISCUSSION

The words fertility and sterility are blanket terms, covering a wide variety of conditions and descriptive of the degree of activity of a physiological function. The terms do not stand for an allelomorphic

pair of genes. We may suppose that every species has achieved, during the course of evolution, a characteristic mechanism for propagating its own kind that has sufficed to effect its survival. This process of normal reproduction entails numerous complexities, the omission or dislocation of any one of which might bring about sterility. In many cases the sterility might be accidental and not transmissible from parent to offspring. On the other hand, it is evident that a large number of either physiological or morphological differences affecting the formation or growth of the germ cells, or the mechanism connected with their liberation or fertilization, might upset the normal function of reproduction and be dependent upon some chemico-physical basis in the germinal constitution which would be passed on from one generation to the next as a true hereditary factor. Any departure from the normal may alter the balance of the reaction either towards an improvement over the condition ordinarily maintained or towards a less favorable one. If "fertility" expresses the normal condition, definitions may readily be found for such terms as "high fertility," "partial sterility," etc., the normal always being retained as the standard of comparison. In a case like fused, the two conditions found in the female of complete sterility with its own males and partial fertility with males from other strains are probably both the manifestations of a single determining cause in the germ cell. Here, the number of individuals which reproduce and also the number of offspring from each individual, is affected.

At present, it is not possible to state definitely whether the determining causes for the cases reported in this paper, consist of separate genes which have sterility as their principal somatic expression or whether they are an integral part of the genes responsible for the associated character, but the latter seems probable. It is evident that we are dealing with the activity of some factor and not concerned with inbreeding, with heterozygosity or the proportion of dominants present, all of which conceptions have been proposed as explanatory of various instances of sterility or fertility.

The sterility of three of the mutants reported here, rudimentary, fused, and reduced bristle, is evidently of the same type and may be elucidated by reference to the same theory. Morula, dwarf, cleft and dibro present no contradictory features. The fact that cleft is sterile in the male sex is readily harmonized with our theory on the assumption that the injurious influence of the cleft gene in the heterozygous female is so severe that it extends to the males produced by her, thus making them incapable of producing offspring.

The most clear-cut case is that of "fused" and a detailed inquiry re-

veals something of the mode of operation of the sterility connected with it. We have seen that the influence that causes the sterility is progressive, not acting with finality upon all of the gametes at any one point, but making itself felt throughout development. At two points in the process it is capable of being counteracted by its normal allelomorph. The harmful reactions (or inhibition of proper activities) set up by the gene, operate during oögenesis, in the stages previous to maturation and result in the production of a very small number of eggs unless their effect is checked by the presence of the normal allelomorph in the maternal chromosomal complex. In case the normal allelomorph is absent during prematuration, the few eggs laid perish unless the sperm which fertilize them bring in the normal allelomorph and restore to a certain percentage of them the usual course of development. The result will be that some of the females will produce a small number of offspring. When a sex-linked factor, such as fused, is concerned, the allelomorph is contained only in the X chromosome, i.e., the female-forming type of sperm, and therefore the F_1 from a fused female results in females only. In the case of a non-sex-linked factor, like reduced bristles, either sperm may carry the normal allelomorph in the autosome so that both sexes are comprised in the filial generation. On the same principle, morula and dwarf would be expected to yield some F_1 males if larger numbers were involved.

Rudimentary does not afford such a clear-cut case as fused but analysis of the data shows that the same principles are at work as in fused although the harmfulness of the latter gene is greater than that of rudimentary. A few rudimentary males may be formed when rudimentary females are fertilized by normal males and the cross of rudimentary by rudimentary is sometimes partially successful. But that the difference between rudimentary and fused is one of degree rather than of kind justifies us in including them in the same general category.

An interesting comparison, though not an exact parallel, in regard to the possibility of a prematuration influence, is afforded by the case of self-sterility in *Nicotiana*, reported by EAST and PARK (1917). The non-occurrence of self-fertilization is explained by reference to the fact that male and female gametes have developed in the same individual and therefore under the influence of identical factors. Incompatible crosses are due to "the likeness of the parents in the effective hereditary factors postulated." The male gametes themselves may be of different kinds yet none can fertilize eggs from the same—or a similar—source as themselves. The prematuration influence in these plants appears to

be dependent upon the presence in the parental complex of a factor for self-sterility together with an unknown number of additional genes instead of being narrowed down as in the case of fused to the influence of one particular factor. The analogy of the cases does not extend to the conception that the identity of parental constitutions, regardless of the gametic type, is responsible for the phenomena observed but merely that the sterility is determined by activities in the diploid cell prior to maturation.

The experiments with fused have demonstrated that the Y chromosome is beyond the influence of the factors in the X chromosome. Recent considerations on the structure and formation of the Y chromosome, published by MULLER (1918), indicate that it is an accumulation of recessives, the product of degenerative changes in the factors. On this basis, it would not be expected to contain a gene in the locus corresponding to that of fused in the X chromosome that would be even remotely related to it. The behavior of the Y chromosome here, showing that it cannot be influenced by fused nor counteract the influence of fused upon other parts of the cell, is in accord with MULLER'S work.

While an exhaustive search for sterility factors is still to be completed, certain facts concerning the nature of the element at work have been pointed out, whether these peculiarities are to be regarded as dependent upon the presence of a distinct entity, the sterility factor—or factors—or whether they are but additional manifestations of the mutant genes themselves. All of the cases investigated appear to belong to the same type, the most clearly defined instance being that of fused. The corroborative evidence furnished by the latter suffices to rehabilitate the discarded theory of prematuration and although the principle of repugnance has not been substantiated an additional one has been suggested, namely, that of restoration.

SUMMARY

1. Males of the following races are fertile: fused, rudimentary, morula, reduced bristle, and dwarf. Cleft males are sterile.
2. Of the few individuals of dibro which were tested, both males and females were sterile.
3. When mated with males of their *own* race, fused and morula females are sterile, rudimentary females are partially so.
4. When mated with males of *other* races, a certain percentage of morula, dwarf, reduced bristle, rudimentary or fused females may give a few offspring; in a few cases morula and dwarf produced a very small number of individuals, all females; occasionally reduced bristle may

reproduce, giving a small number of each sex; rudimentary may yield a larger number of individuals, with females predominating; from 413 fused females, 1628 females and no males (except a few produced through non-disjunction) have been obtained.

5. In no case has it been possible to isolate a factor for sterility independent of the mutant factor itself. In each case the sterility shown is, therefore, probably one of the manifold effects of the gene responsible for the mutation.

6. The sterility involved decreases not only the number of fertile individuals in each race but also the number of offspring produced by each fertile female. However, the harmful effect of the mutant gene upon the egg may be counteracted at two points in the history of the gamete: (1) in the prematuration stages of oögenesis, that is, if the female is heterozygous for the mutant gene, a larger number of individuals will be produced than if she is homozygous; (2) if, at the time of fertilization, the sperm brings in the normal allelomorph, even though the female is homozygous and the number of eggs consequently reduced, a larger number of offspring will result than when the sperm carries the mutant gene. The Y chromosome has no corrective influence.

7. No "repugnance" has been observed between the gametes forming the recessive mutant classes.

I wish to acknowledge my indebtedness to Professor T. H. MORGAN for his helpful criticisms during the course of these investigations. I wish also to express my appreciation of the valuable suggestions of Dr. C. B. BRIDGES and Dr. A. H. STURTEVANT.

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ON VARIATION IN TARTARY BUCKWHEAT, *FAGOPYRUM TATARICUM* (L.) GAERTN.¹

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INTRODUCTION

From the morphogenetic point of view the manifestation of dimorphism in certain races of plants,—the so-called ever-sporting varieties,—presents a very interesting problem. The remarkable feature of these races is the constancy with which the two diverging forms of the same organ are transmitted in ever-sporting fashion; no breeding method has, as yet, been conceived by which, for instance, certain variegated types of plants or certain strains of *Matthiola*, could be induced to breed true. These races appear as compound forms ever transmitting the potentialities of the two component types.

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Dimorphism manifests itself in two externally different forms. The different, "mutually exclusive" characteristics may appear simultaneously distributed in the organs of the same individual as in *Trifolium pratense quinquefolium*, *Veronica agrestis*; in another group of plants each individual of the race may display only one of the dimorphic characters as in the case of certain strains of *Matthiola*, *Antirrhinum*, *Dipsacus sylvestris torsus*, etc.

In interpreting these phenomena, DE VRIES, whose investigations involved a great abundance of material, assumes the peculiar behavior of these races to be due to the interaction of two "antagonistic, mutually exclusive, characters." The operation of these two contending characters within the individual leads to the formation of two distinct groups of plants, the half-races and the middle races or ever-sporting varieties. Opposed to this interpretation is the view held by certain writers who consider the ever-sporting nature of many of these races as mere somatic variations and relegate them into the group of non-heritable modifications.

More recently, however, some of the ever-sporting types in plants as well as in animals have been subjected to a genetic analysis and their peculiar mode of inheritance has been explained in terms of Mendelian factors.

The purpose of the present publication is to record the results of a study on a highly variable, ever-sporting race which the writer has discovered in *Fagopyrum tataricum* Gaertn. (*Polygonum tataricum* Linn.). This race was isolated in 1916 and five generations have been grown since. In the course of his observations on this race the writer's attention was chiefly devoted to the study of variation and transmission of the external characters in an endeavor to establish first by direct experiment the hereditary behavior of this race under different conditions before attempting an analysis of the underlying genetic causes. The study of the phenotypic expression of the characters of this new race has furnished enough interesting data and observations to warrant their publication.

MATERIAL AND METHODS

The race with which this paper is concerned originated from commercial fruits of *Fagopyrum tataricum* Gaertn., Tartary buckwheat, also known as India wheat, which had grown in Maine. While examining these fruits preparatory to planting in the spring of 1915, the writer's

attention was attracted by a rather high number of fruits whose structure differed from the normal triangular type in that they possessed a quadrangular and, some of them, a quinquangular form. In the spring of 1915 these abnormal fruits were planted along with several hundreds of normal fruits in rows in the cereal-breeding garden of this STATION at Aroostook Farm. The rows were one foot and a half apart and the individual plants stood 3 inches apart within the rows. To guard against any possible interference on the part of insects the plants were covered with cheese-cloth screens.

Frequent examination of the flowers borne by each of the plants originated from the abnormal fruits in the summer of 1915, revealed a high degree of variability in the number of carpels. Deviation from the normal number of perigone members was also noticed. Each of these plants was harvested separately and the fruits examined. The plants showed a varying degree of variability in the number of abnormal fruits. The precise ratio between the number of normal and abnormal fruits could not be established, as some of the fruits had been lost before the plants were examined. However, in all cases the number of abnormal fruits did not equal that of the normal ones. One plant was found to be distinguished by a particularly high degree of variability in the shape of its fruits. This plant was selected as a starting point of a strict pedigree culture in 1916. The strain descended from this original plant will be referred to here as line 5.

For the purpose of comparative study, an apparently normal strain of Tartary buckwheat designated in our record books as line 22 was selected in the same year and grown ever since along with the highly variable line 5 under the same environmental conditions, as a control line.

In order to test these strains in as many generations as possible in the course of a few years the development of these races was continued over the winter in the greenhouse. This afforded the further advantage of permitting the study of the race under different conditions of environment. As will be seen later certain environmental conditions have a very marked influence upon the range of variation of the characters of this race. Indeed, the regularity and uniformity of response of the cultures to certain conditions of environment seem to throw some light upon the releasing agents that are involved in the manifestation of the variation and in the control of its scope in this race. A description of the different conditions of environment will be given later in connection with the analysis of the data on each generation.

The methods used in the examination of the material and in recording the data may here be briefly outlined. The variability of the race in question affects the number of carpels and perigone leaves. In regard to recording the number of carpels two methods have been used. The first one, the simpler of the two, consisted in the examination of all fruits of each plant and in their distribution in groups according to the number of angles of the fruits. As the angles of the buckwheat fruit correspond to the midribs of as many carpels, the number of angles of a fruit is equivalent to the number of carpels of the flower from which the fruit had originated. After sorting the different fruits into groups the individuals of each group were counted and the ratio of the different groups established. Over 44600 fruits were recorded by this method.

This method, while relatively simple and convenient, does not reproduce an absolutely exhaustive and truthful picture of the entire range of variation. For, as will be seen later, the more extreme variants of this race, the flowers with higher numbers of supernumerary carpels, are sterile. Consequently the statistical examination of the ripe fruits does not reveal the entire range of variation which by neglecting the extreme variants, i.e., the sterile flowers with a high number of supernumerary carpels, is manifestly narrowed down to forms more closely approaching the normal type. However, the extreme variants are rather rare and their exclusion from consideration affects the ratio between the total numbers of normal and abnormal flowers to only a slight degree. A complete picture of the range of variation embracing the full amplitude of the aberrant forms is obtained by the laborious method of picking and examining the flowers successively as they appear on the plant. The difficulty of this method is at once obvious if the smallness and structure of the flowers of *Fagopyrum tataricum* are recalled. In order to determine the number of carpels, each flower was examined with the aid of a hand lens and the number of stigmas counted. In connection with the study of the distribution of abnormal flowers upon the plant as well as for the purpose of determining the complete range of variation of this race, over 12000 flowers were examined by this method. In all, upward of 57000 flowers and fruits were examined in the study of this race.

Before considering the data it seems advisable to give a description of the morphology of the normal and abnormal flowers of this species.

DESCRIPTION OF THE NORMAL AND ABNORMAL FLOWER

The flowers of *Fagopyrum tataricum* are normally borne on axillary simple racemes. The normal flower is typically trimerous except the perigone, which consists of a whorl of 5 similar green leaves with a yellowish tinge. The androecium is composed of two whorls of stamens. The outer whorl bears five leaves which according to EICHLER (1876-1878) developed from an originally trimerous whorl by reduplication of two of its members. The inner whorl consists of three stamens. In contrast to the dimorphic heterostyly of the common buckwheat, *Fagopyrum esculentum*, the flowers of Tartary buckwheat are homostyled, the relation between the height of the stamens and pistils being the same in all individuals of the species. The stamens of the outer whorl are usually incomplete with anthers scarcely developed. They seem to play no part in the pollination process. This is entirely left to the three stamens of the inner whorl. In examining such large numbers of flowers the writer invariably found the pistil to be very closely surrounded by the inner stamens; their anthers adhere to the stigmas of the carpels and upon bursting cover them with pollen, thus insuring self-fertilization of the flower. This self-fertilizing mechanism of the flower of *Fagopyrum tataricum* makes this species far better adapted to the study of inheritance of its characters than the heterostylous *Fagopyrum esculentum*, since it offers no difficulty in raising pure-line cultures.

The gynoecium is normally composed of three carpels which unite to form a triangular ovary. Each carpel is only slightly prolonged into a very short style terminating with a stigma. The normal fruit is a triangular achene or nutlet (figure 1, first fruit in upper row).

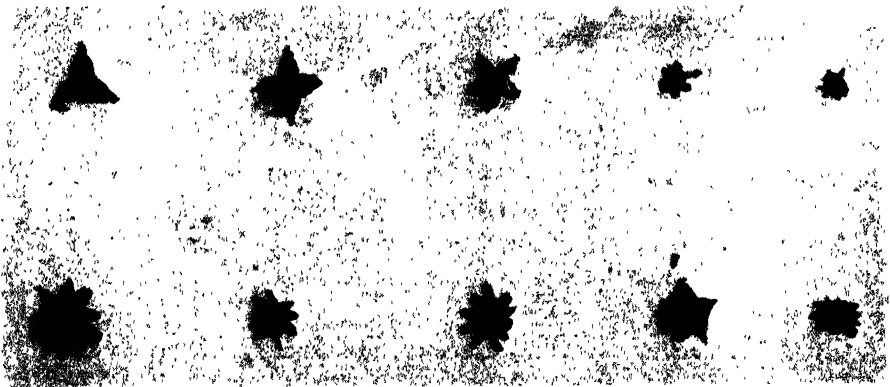


FIGURE 1.—Fruits of an ever-sporting strain of *Fagopyrum tataricum* with supernumerary carpels varying in number from 3 to 12. $\times 1.8$.

The abnormal flowers occurring in this race show a great diversity of forms. The abnormal condition of the flowers affects chiefly two floral organs, the gynoecium and the perigone. It also affects the number of flowers per individual pedicel as in the case of the so-called synanthous flowers. The characteristic feature of all abnormalities occurring in this race is that they represent deviations from the normal extending only in the plus direction.

Before describing the main forms of the abnormal flowers we may note the scant references recorded in the teratological literature regarding variation in the flowers of *Fagopyrum tataricum*. It may be stated in advance that a careful survey of the literature revealed only a single reference regarding variation in the number of carpels of *Fagopyrum tataricum*. PULLMAN (1905, p. 11), in his account of observations on *Fagopyrum esculentum*, calls attention to the occurrence of flowers with more than three carpels giving rise to achenes with four, five and more angles. In this account he makes a brief statement that "many-angular fruits also occur in *Fagopyrum tataricum*." No other record regarding abnormalities in the gynoecium of *Fagopyrum tataricum* has come to the writer's notice. Nor are references concerning the abnormalities in the gynoecium of *Fagopyrum esculentum* and in the Polygonaceae in general, to be found more frequently in the literature. While PULLMAN makes the first direct reference regarding supernumerary carpels in the gynoecium of *Fagopyrum*, the phenomenon was known, though not recognized as such, as far back as 1829 when LOISELEUR and DESLONGS-CHAMPS (cf. PENZIG 1894, p. 265) found an unusually enlarged ovary of *Fagopyrum esculentum* which they erroneously described as a new species, *Polygonum pyramidatum*. This was undoubtedly a syncarpous gynoecium formed by supernumerary carpels. WEBER (1860, p. 365) mentions generally the occurrence of an increased number of carpels in *Polygonum*. BENTHAM (1865, p. 714) describes the flower of *Polygonum* as having 2 or 3 styles, the fruit being, accordingly, flattened biangular, or triangular. Further references regarding the abnormalities in the gynoecium of the different forms of *Polygonum* sp. are found in PENZIG's "Pflanzen-Teratologie." Thus in *P. persicaria* the style is 2-3 cleft, the achene bi- or triangular. In *P. orientale* a gynoecium has been observed with two whorls of carpels. CLOS (cited by PENZIG 1894, p. 265) mentions a case of adesmy of the carpels in the same species. In the gynoecium of *P. tinctorum*, two instead of the normal number of carpels occur.

While it is of interest to note these instances of instability in the structure of the gynoecium in the different forms of *Polygonum*, it is equally obvious that these deviations from the normal type occur only very rarely and that their range of variability is very limited. Concerning the variation in the number of perigone leaves, the writer is not aware of any previously recorded observation in regard to *Fagopyrum tataricum* or *Fagopyrum esculentum*, though a few instances of such variations have been noted in other forms of the Polygonaceae (cf. WEBBER 1889).

The abnormal flower form most frequently encountered in our race possesses a gynoecium composed of 4 carpels giving rise to a quadrangular achene. Frequently associated with this quadri-carpellate gynoecium is a perigone consisting of six instead of five leaves. As will be noted later, over 25 percent of all examined four-carpellate flowers bore six-parted perigones. Less frequently occurring are the five-carpellate flowers of which about 24 percent were found to bear six-parted perigones. Flowers with 6-10 carpels occur fairly frequently in this race. Flowers with 11, 13, and 15 carpels are of rare occurrence. A few flowers were observed with as high a number of carpels as 19 and 20. One flower was noted with a gynoecium composed of 25 carpels and bearing a perigone of 18 leaves. This flower marked the most extreme variant observed in this race. The number of supernumerary members of the perigone varies up to 14 although a few flowers were found with 16 perigone leaves. Eighteen perigone members were the highest number observed. Figure 1 presents a series of abnormal fruits originated from flowers with 4-12 carpels taken from one plant. Figure 2, A, B, C, represent flowers with 6, 7, and 8 perigone leaves.

As the number of supernumerary carpels increases their frequency decreases. In a flower with 8 or more carpels the shape of the gynoecium appears modified. The gynoecium loses its angular structure, the numerous, crowded carpels make it appear larger and more globular presenting on cross-section a wavy circular line. Very often these floral monstrosities show at the apex an aperture of varying width (see figure 3, A). In some extreme cases the ovary is entirely open, forming at the top a more or less elliptical ridge which is lined up with the stigmas of the numerous carpels. As a rule the flowers with over 7 or 8 carpels are sterile, but very frequently the ovary of these flowers increases considerably as seen in figure 1, lower row, and assumes the appearance of a developed fruit, only to wither within a short time.

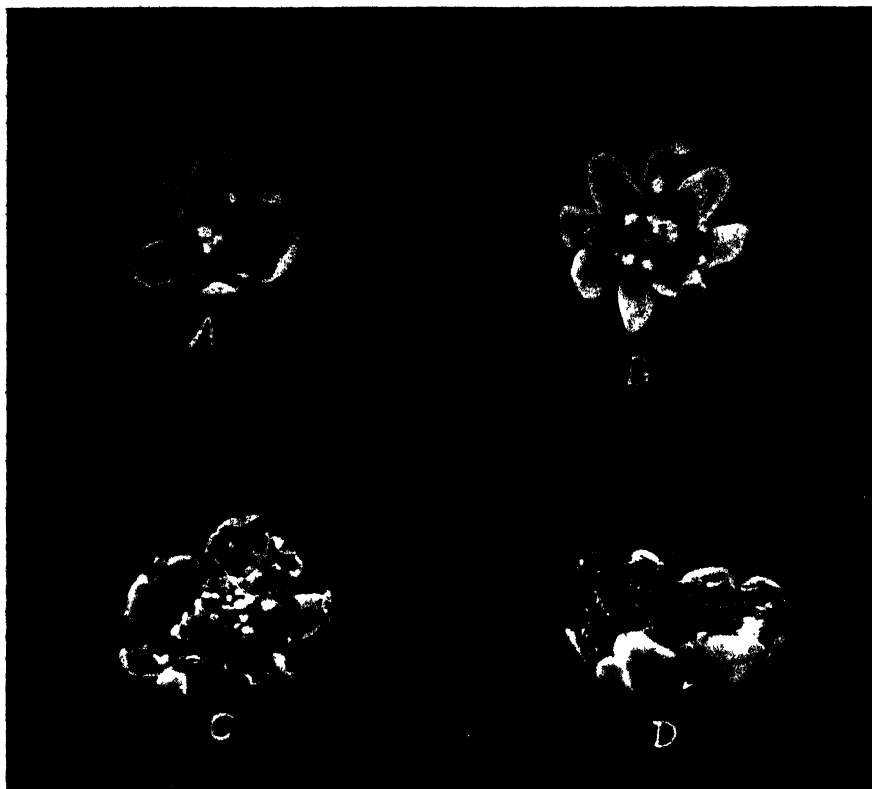


FIGURE 2.—Abnormal blossoms of *Fagopyrum tataricum*.—A. Flower with six-parted perigone. B. Flower with eight-parted perigone. C. Flower with seven-parted perigone. D. A case of syncarpy. The fruit to the right is normal, triangular, the one to the left is abnormal, five-angled. The stigmas of the carpels are still noticeable at the summit of the fruits. All $\times 5$.

Before closing the description of the abnormal flowers attention may be called to another abnormality which hitherto has not been recorded for *Fagopyrum tataricum*. While in the abnormal flowers, considered above, the gynoecium bears the semblance of a single pistil, in the cases to be now considered, the flowers show two or three distinct pistils. Two forms of these abnormal flowers can be distinguished. The torus of the flower may appear unusually enlarged, and on its surface two or three distinct pistils may appear. All the pistils, however, are surrounded by a common perigone composed of numerous leaves. These flowers are naturally large and can be easily detected on the plant even in bud condition.

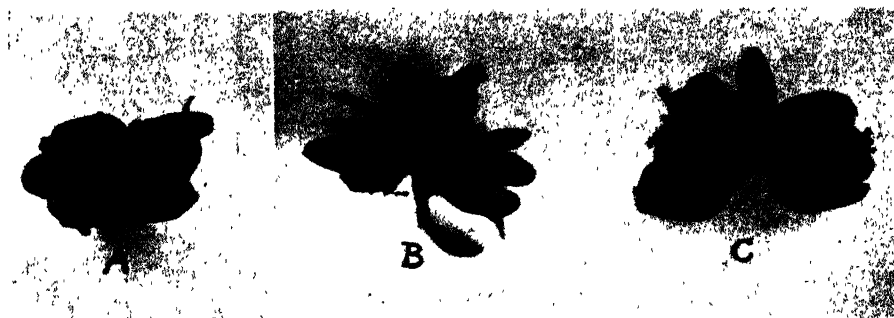


FIGURE 3.—Abnormal blossoms and fruits of *Fagopyrum tataricum*. A. A monstrous fruit with 12 carpels, viewed from above. The carpels did not fuse at the summit, leaving a distinct, elliptical aperture. This type of fruit is invariably sterile. B. An interesting illustration of synanthous development involving 3 flowers. C. A case of synanthry involving two highly abnormal flowers. Each flower appears surrounded by a separate perigone. All $\times 5$.

The second form, more frequently met with, presents a structure known as "synanthry." Two or three complete flowers are borne on a single, broadened pedicel and are surrounded by separate perigones. Synanthous flowers with two pistils (figure 3, C) occur rather often in this race while synanthies involving three flowers as shown in figure 3, B, are of rare occurrence. Regarding the structure of the flowers forming the synanthies it is of interest to note that, as a rule, they are themselves abnormal in that their gynoecium is composed of more than three carpels. In a few rare cases where one member of the synanthry developed a normal triangular pistil the other member was invariably found to possess abnormal pistils composed of more than three carpels. In cases where the number of carpels in the gynoecium of the synanthous flowers was comparatively low the flowers often developed to maturity giving rise to syncarpous fruits (figure 2, D). A more detailed account of the different forms of synanthry occurring in this race will be given in another paper.

ANALYSIS OF DATA

First generation

The data to be considered here will be presented in chronologic sequence in connection with each of the five generations of this race.

The first generation of the original plant selected in 1915 was grown in the greenhouse of the UNIVERSITY OF MAINE in the winter of 1916. In order to determine the effect of nutrition upon the production of ab-

normal flowers, the fruits were subjected to different cultural treatment. A part of the fruits was planted in an 8-inch pot, No. 2, filled with normal garden soil which had been mixed with ordinary commercial fertilizer. In the second series the other portion of the fruits was planted in pot No. 2a containing soil that had been mixed with a considerable amount of sand.

Regarding the other conditions of environment, they were alike for the cultures in both pots. The temperature of the greenhouse was kept at 70-75 degrees Fahrenheit (21-23.8° C.).

The examination of the flowers as they appeared on the plants revealed a high degree of variability in the structure of the gynoeceum and perigone. In table 1 are given the frequencies of the different flower forms found on the plants grown in fertilized soil, in pot No. 2.

TABLE I
LINE No. 5.—*Frequency distribution of the number of flowers with respect to the number of carpels.*

Plant No.	Number of carpels											Synanthies	Total
	3	4	5	6	7	8	9	10	12	14	16		
1	33	119	15	4	1	3	1	1	1			2	180
2	39	147	31	13	2	8			2		1	3	246
3	58	222	51	17	1	4	4	5	3	1		6	372
4	35	117	22	3			1	2			1	1	181
5	40	126	28	10	2		1	2				3	213
Total	205	731	147	47	6	15	7	10	6	1	2	15	1192
Percentage	17.21	61.33	12.33	3.94	0.50	1.26	0.59	0.84	0.50	0.08	0.17	1.26	

From these figures it is evident that the number of abnormal flowers is far in excess of that of the normal ones, the percentage ratio being 17.21 : 82.79. The flowers with four carpels show the highest frequency, constituting 61.33 percent of all flowers and 74.06 percent of all abnormal flowers. The percentage frequencies for all five plants are graphically represented by the solid-line curve in figure 4. It is a bilateral curve whose apex is determined by the four-carpellate flowers. The short left flank of the curve is determined by the normal flowers while the abnormal variants form the extended right flank of the curve.

In examining the figures in table 1 and the corresponding curve in figure 4, it may be noted further that while the frequency distribution as a whole decreases with the increasing number of carpels per flower, the flowers with an even number of carpels occur more frequently than

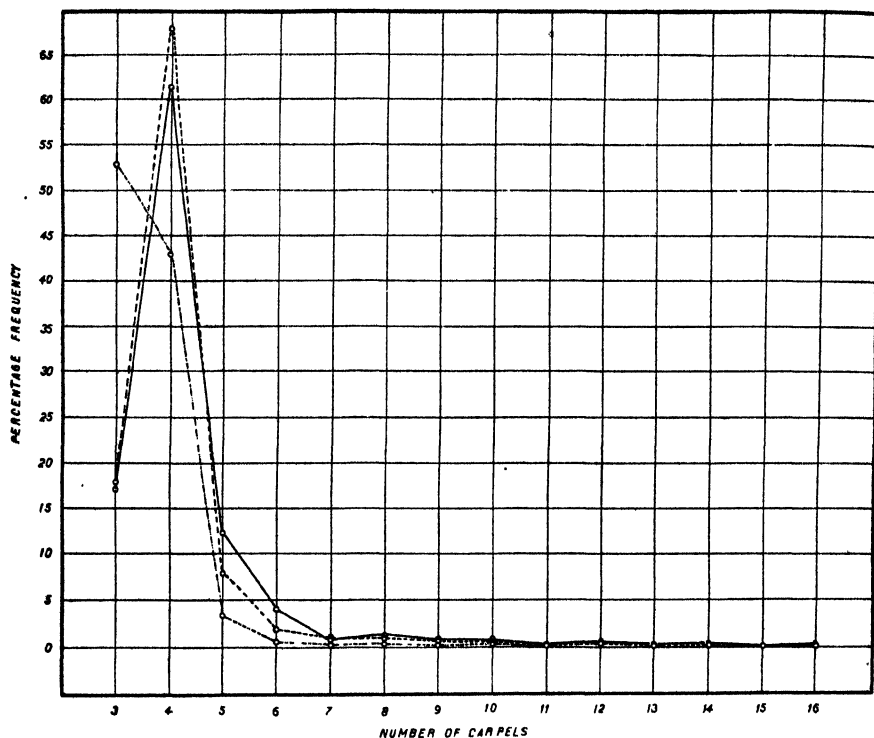


FIGURE 4.

those with an odd number of carpels from the six-carpellate flower on. Flowers with 11 and 13 carpels are of very rare occurrence while flowers with 15 and 17 carpels have not been observed either in this or in the following generations. To be sure, the number of flowers in the higher polycarpellate categories is small but it is of interest to note that this tendency of the anomalies to produce more pistils of an even number of carpels than those of an odd number has been maintained in all generations of this race in which the more extreme anomalies have been produced. This tendency, as will be clearly seen from figure 4, interferes with the regularity of the curve of variation.

Associated with the variation in the number of carpels in the flowers of this race is a comparatively moderate degree of variability in the number of the perigone leaves. At the time when the data on the first generation were collected the variations in the perigone were also noted, but the data do not represent the exact numerical relationship between the different categories of the perigone forms as they were not recorded

until sometime later when it was discovered that their occurrence was more than accidental. It is in connection with the study of the fourth generation of this race that an exact statistical examination of the variations in the perigone was made. The data as they were recorded for the first generation are given in table 2 in totals for all the five plants.

TABLE 2

Number of perigone leaves.....	5	6	7	8	9	10	16	Total
Number of flowers.....	1053	86	25	5	4	3	1	1177
Percentage	89.59	7.21	2.11	0.42	0.34	0.25	0.08	100

These figures clearly show that the frequency distribution of the flowers with the normal five-parted perigone prevails decidedly over those with abnormal perigones, the percentage ratio being 89.59:10.41. Among abnormal perigones the six-parted perigone shows the highest frequency, 69.35 percent of all abnormalities. In accordance with numerous observations of DE VRIES (1910), HARRIS (1917), and other writers, the frequency distribution in this case decreases with the increasing degree of the deviation. This variation can be represented in the form of a skew or unilateral curve whose apex is formed by the normal five-parted perigone of the species.

The plants that grew in the unfertilized sandy soil were found to behave very much like the plants considered above. The statistical examination of these plants, 15 in all, with regard to the variation in the gynoeceum, gave the results shown in table 3.

TABLE 3

Number of carpels	3	4	5	6	7	8	9	10	11	12	13	20	Total	Synanthies
Frequency ...	437	1436	300	63	8	18	9	10	3	6	1	2	2293	28
Percentage ..	18.83	62.62	13.08	2.75	0.35	0.79	0.39	0.44	0.13	0.26	0.04	0.08		1.22

A comparison of these percentage frequencies with those given in table 1 for the plants grown in fertilized soil shows that the behavior of the plants in both series in regard to the distributions of the corresponding categories of flowers is practically identical. The difference in the media in which the plants grew affected neither the numerical relationship between the different classes of variants nor the range of the variations.

Very similar conditions in both series of plants were found to prevail in the case of the variations in the number of perigone leaves. Table 4 gives the frequency distribution of the flowers with respect to the number of perigone leaves in the 15 plants grown in sandy soil in pot No. 2a.

TABLE 4

Number of perigone leaves	5	6	7	8	10	12	13	14	18	Total
Frequency	1994	217	43	17	5	4	1	1	1	2293
Percentage	87.13	9.45	2.28	0.73	0.22	0.17	0.04	0.04	0.04	100.00

It will now be of interest to turn our attention to the first generation of plants originated from fruits of the plant that appeared originally as a normal representative of the species as it had not produced any other than triangular fruits. The first generation raised from these fruits comprised 13 plants which grew alongside the first generation of the variable race considered above. The flowers of all the 13 plants were examined in the same manner as those of the variable race with the results given in table 5.

TABLE 5

LINE No. 22.—Frequency distribution of flowers with respect to the number of carpels.

Plant No.	Number of carpels					Total
	2	3	4	5	6	
1		165	4			169
2	1	140	10		1	152
3	1	189	14	1		205
4		76	8			84
5		68	4			72
6	1	73	3			76
7		131	4			135
8	1	62	3			66
9		52	4			56
10		37				37
11		23	1			24
12		43				43
13		27				27
Total	4	1086	55	1	1	1147
Percentage	0.35	94.68	4.79	0.09	0.09	100

From table 5 it is evident that the 13 plants of line No. 22 exhibit an entirely different behavior from that of the variable line No. 5. The normal flowers of the species constitute in line No. 22 the bulk of the

flower output while the abnormal variants represent only 5.32 percent of all the flowers. With regard to the variations in the perigone leaves, 29 flowers out of a total of 1147 or 2.53 percent developed a six-parted perigone. While this strain may well represent the normal, slightly variable type of the species, its behavior under greenhouse conditions suggested a similarity with a number of strains which DE VRIES has named "half-races" in distinction from the ever-sporting varieties.

Second generation

In order to examine the behavior of the characters of these two lines, a limited number of flowers on the plants of the first generation were marked, to avoid repetition in counting, and permitted to mature. The matured fruits were harvested from each plant separately and planted outdoors in the spring of 1916. The fruits from plant 5, pot 2, of the ever-sporting line No. 5 and from plants No. 3 and 7 of pot 4, of line No. 22 were subjected to different cultural treatment. The fruits of each plant were divided into two parts; one portion was planted in pots (Nos. 15, 16 and 17) containing only gravel, while the other portion of the crop was planted in pots (Nos. 18, 19 and 20) filled with rich soil mixed with commercial fertilizer. A number of fruits from other plants of the first generation were planted in pots (Nos. 22, 23 and 24) filled with normal soil. All the pots were placed outdoors. The data on the variation of these plants were obtained by determining the number of angles of each fruit and then grouping the fruits into the different categories. In table 6 are given the data for the second generation of line No. 5.

A comparison of these figures with those on the first generation presented in table 1 shows a striking difference in the range of variation and in the numerical relationship between the different variants. The average percentage ratio between the normal and abnormal fruits in all the second-generation plants of this race is 62.85:37.15 as against 17.21:82.79 in the first-generation plants. No less affected is the range of variation which is narrowed down to six-angular fruits, i.e., six-carpelled flowers, as the most extreme variants. Thus our race under the conditions influencing the growth of the second generation not only failed to progress in the development of its character, but showed a very distinct reversion toward the normal type of the species.

The data on the second generation of the very slightly varying line No. 22 are given in table 7.

TABLE 6

LINE No. 5.—*Actual (f) and percentage frequencies of numbers of fruits with respect to their number of carpellary angles.*

Pot No.	Source of fruits	Medium	Number of fruit-angles								Syncarpies	
			3		4		5		6			
			f	percent	f	percent	f	percent	f	percent	f	percent
17	Plant 5, pot 2	Gravel	486	66.30	235	31.77	10	1.36	1	0.14	3	0.41
20	Plant 5, pot 2	Rich soil	864	66.72	295	30.50	34	2.62	1	0.08	1	0.08
22	Plant 4, pot 2	Normal soil	295	63.58	164	35.34	5	1.08				
23	Plant 3, pot 2	Normal soil	187	61.51	110	36.19	7	2.30				
24	Plant 10, pot 2	Normal soil	687	56.13	459	37.50	78	6.37				

TABLE 7

LINE No. 22.—*Actual (f) and percentage frequency of number of fruits with respect to their number of carpellary angles.*

Pot No.	Source of fruits	Medium	Number of fruit angles			
			3		4	
			f	percent	f	percent
15	Plant 3, pot 4	Gravel	222	99.10	2	0.90
19	Plant 3, pot 4	Rich soil	1839	99.73	5	0.27
16	Plant 7, pot 4	Gravel	175	100		
18	Plant 7, pot 4	Rich soil	1491	99.46	8	0.54
21	Plant 10, pot 4	Normal soil	470	100		

When compared with the data on the first generation given in table 5, these results show, as in the case of the ever-sporting line No. 5, that the conditions under which the second generation plants grew, narrowed the range of variation and favored the development of the normal type of flower or fruit. The average percentage ratio between the normal and abnormal fruits of the second generation plants is 99.66:0.34 as against 95.01:4.99 in the first generation.

Third generation

The third generation of the two strains was grown in the summer of 1917 under three different sets of environmental conditions. In the first series the fruits were planted in pots containing fertilized rich loam of Aroostook County, Maine, and were placed in the greenhouse at the AROOSTOOK FARM. The second series comprised plants originated from fruits that had been planted in pots filled with sand and gravel and placed outdoors. In the third series the fruits were sown in the open

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LINE No. 5.—Actual (f)

Pot or row No.	No. of plants	Source of fruits	Medium and
Pot 28	6	Plant 1, pot 20	Rich soil, gre
Pot 35	13	Plant 1, pot 20	Sand, outdoor
Row 713	2	Plant 1, pot 20	Garden
Pot 30	4	Plant 1, pot 17	Sand, outdoor
Row 715	2	Plant 1, pot 17	Garden

LINE No. 5.—Actual (f)

Plant No.											Total	
	3		4		5		6		7			
	f	percent	f	percent	f	percent	f	percent	f	percent		
Pot 36	1	78	17.69	320	72.56	27	6.12	5	1.13	2	0.45	441
	2	49	14.41	249	73.24	17	5.00	10	2.94	1	0.29	340
	3	119	19.97	389	65.27	46	7.72	10	1.69	3	0.50	596
Pot 37	4	130	16.62	520	67.01	66	8.51	14	1.80	5	0.64	776
	5	136	20.80	430	65.60	51	7.80	8	1.22	6	0.92	654
	6	150	21.08	488	68.54	49	6.88	7	0.98	3	0.42	712
Pot 39	7	127	15.05	572	67.77	67	7.94	24	2.83	9	1.07	844
	8	190	17.48	719	66.51	101	9.34	15	1.39	6	0.56	981
Total		979		3687		424		93		35		444
Percent		17.98		67.73		7.79		1.71		0.64		

ground in the cereal garden. It should be noted that the fruits of each individual plant selected for the propagation of the race, were subjected to the three different cultural treatments.

In table 8 are given the summarized results obtained from the examination of the three series of plants of the third generation of the ever-sporting race.

From these figures it will be noted that the third generation of the ever-sporting variety also displayed a type and range of variation different from that of the first generation. The figures taken as a whole in conjunction with the data, not given in the above table, on a number of plants grown in the garden, indicate that the variation is unilateral, with the normal type of flowers prevalent over the abnormal flowers, though not so distinctly as in the second generation.

If we may assume the variation manifested by the plants grown in the garden to be typical of the third generation, the ratio between the normal flowers and the total of abnormal flowers was found to be 52.78:47.22. The dash-and-dot curve in figure 4 illustrates the variation in the type of fruits of the third-generation plants grown in the garden.

The results obtained from the examination of the third generation of line No. 22 may be stated briefly. The series grown in pots filled with sand and gravel and kept outdoors comprised 23 plants which developed only triangular fruits, 381 in all. The data on the series grown from the same fruit fraternity in garden rows were based on the examination of 10 plants that yielded a total of 3191 fruits. Of these 99.47 percent were triangular, 0.44 percent four-angular, and 0.09 percent five-angular, fruits.

From a consideration of the three generations thus far analyzed it is evident that the variations shown by the second and third generation are significantly different from those of the first generation, notwithstanding the fact that in many points, notably the nutritional conditions prevailing in the second and third generation, the cultural treatment was very much like that in the first generation. This pointed strongly towards the existence of certain specific conditions prevailing in the greenhouse of the UNIVERSITY OF MAINE, where the first generation had grown, that might have operated in a manner favorable to the development of abnormal flowers in this race. With a view to testing the influence of these conditions the fourth generation of both races was grown again in the UNIVERSITY greenhouse, in the winter of 1917-'18, under conditions as nearly identical with those of the first generation as possible.

Fourth generation

The bulk of the fourth-generation plants originated from fruits borne by plants that had grown in the summer of 1917, in pot No. 26 in the greenhouse at the AROOSTOOK FARM, and which had yielded 2754 fruits, 48.17 percent of which were triangular, 46.07 percent four-angular, 5.73 percent five-angular, and 0.04 percent six-angular. The kind of fruits and nutritional stratum for the raising of the fourth generation were selected in a manner that promised to furnish some evidence relative to a few problems that have developed in the course of the study of line No. 5. For the continuation of this race from generation to generation four-angled fruits were chiefly used, but in some cases, where three-angled fruits were used, it was noted that their offspring did not differ markedly from that raised from four-angled fruits of the same plant. It was thought desirable to obtain some evidence on this question by the examination of the material furnished by the fourth generation. In connection with the study of the fourth generation an attempt was also made to determine the periodicity, if any, in the occurrence of abnormal flowers or fruits of this race at different flowering periods of the plant. This problem will be dealt with in a separate section of this paper. Finally, the inquiry into the effect of optimum and deficient nutrition upon the variation of the flowers was continued in the fourth generation.

With these problems in mind, three-, four- and five-angled fruits of the same fruit fraternity from pot No. 26, were planted separately in three pots, Nos. 36, 37, and 38, respectively. All the three pots had ordinary garden soil as a common growth medium. Another part of fruits of the same crop was planted in pot 39 filled with composted soil, while still another portion of the crop was planted in pots 42 and 48 filled with sand.

The fruits for the continuation of line No. 22 were taken from the third-generation plants that had grown in the summer of 1917 in plot 777, row 738, in the cereal garden, yielding 99.28 percent of three-angular, and 0.72 percent of four-angular, fruits. Some of the three-angular fruits of this plant were planted in pot 46 filled with composted soil. In connection with the determination of the effect of environment upon the variation in the flowers a number of other pot cultures were grown but they may be passed over here as they will be referred to when the question of the influence of environment will be discussed in another section of this account. All the pots were placed closely together on the south side of the UNIVERSITY greenhouse.

Three plants each from pot 36 and 37 and 2 plants from pot 39 were selected for the study of the periodicity in the frequency occurrence of the abnormalities. The flowers of these plants were examined and recorded daily, including Sundays, by the method previously pointed out. The flowers of 2 plants in pot 38 were also examined in the same way, but not recorded daily, as they were not included in the material for the study of the periodicity in the occurrence of the different forms of flowers. The flowers on the remaining plants in each of the pots mentioned above were left unmolested to maturity when their fruits were examined and their different forms recorded. Thus by examining the variability of two sets of plants grown in the same pot and raised from the fruits of the same plant, each by a different method, it could be determined to what extent the method of counting and examining the ripe fruits followed in the analysis of the second and third generation approached the exhaustive picture of variation obtained by the counting and examination of the flowers.

The data collected by examining all the flowers, 5444 in all, on some of the plants in pots 36, 37 and 39, are regarded here as representing the type and range of variation of the fourth generation of this race. The actual and percentage frequencies of the different flowers of the fourth generation plants are presented in table 9.

A comparison of these figures with the data on the first generation given in table 1 at once reveals a striking similarity of type and range of variability in the two generations, thus bearing out the assumption made above. The average percentage distributions of the flowers of the first- and fourth-generation plants, given in the lowest horizontal array in both tables, are practically identical, indeed, they are in more complete accord with each other than the corresponding distributions of flowers on plants grown in the same pot in each of the two generations. While there is a slight variation in the numerical relationship of the abnormal variants of both generations, the percentage ratio between the normal flowers and all abnormal flowers is practically the same, being 17.21:82.79 for the first generation and 17.98:82.02 for the fourth generation. The similarity of the variability in the first and fourth generations is graphically represented by the dashed and solid curves in figure 4.

It may be argued that these highly concordant results obtained for the first and fourth generations as well as the much higher degree of variability of these as compared with the second and third generation, may be due to the fact that in the examination of the first and fourth generation

the method of picking and examining the flowers was used while the results for the second and third generation were obtained by examining the ripe fruits. It is also conceivable that the traumatic stimulation caused by the daily removal of the flowers may have influenced the degree of development of the abnormalities. It will, therefore, be instructive to consider now the results obtained from the examination of these plants from pots Nos. 36, 37 and 39, whose intact flowers were permitted to develop into fruits which were examined at maturity in the same manner as in the case of the second and third generation. Table 10 gives the summarized results for 7 plants from each pot.

TABLE 10

LINE No. 5.—*Actual (f) and percentage frequency of number of fruits with respect to number of peltary angles.*

Pot No.	No. of plants	Number of fruit-angles									
		3		4		5		6		8	
		f	percent	f	percent	f	percent	f	percent	f	percent
36	7	32	23.02	84	60.43	19	13.67	4	2.88		
37	7	25	17.60	95	12.68	18	12.68	2	1.41	2	1.41
39	7	31	15.66	157	79.29	9	4.55	1	0.60		
Total		88		336		46		7		2	
Percentage		18.37		70.15		9.60		1.46		0.42	

The figures in table 10, when compared with those in table 9, clearly show that the results obtained by the examination of the ripe fruits are practically the same as those secured by the examination of picked flowers. It is true that the range of the variation determined by the former method is somewhat limited owing to the failure of the flowers with high numbers of carpels to set fruits, but the percentage ratio between the normal and all abnormal flowers—17.98:82.02—is practically the same as the percentage ratio between the normal and all abnormal fruits 18.37:81.63.

Turning to the question of the behavior of the offspring from normal and abnormal fruits borne by the same plant we may now consider the results obtained for the plants in pots 36, 37 and 38. The data on the plants of the last-named pot have not been included in table 9, as they were not collected daily as in the case of the plants in the other pots. As will be remembered the plants in pot 38 originated from three-angular

fruits, those in pot 37 from four-angular, and those in pot 36 from five-angular fruits. The data on the offspring of the four- and five-angular fruits have been already given in tables 9 and 10, pots 36 and 37, respectively. The data on the 3 plants that had originated from triangular fruits in pot 38 are given in table 11.

TABLE 11

Number of carpels	3	4	5	6	7	9	10	12	16	Synanthies
Number of flowers	64	226	27	7	3	3	1	3	2	8
Percent	18.60	65.71	7.85	2.03	0.87	0.87	0.29	0.87	0.58	2.33

The examination of the fruits of 11 plants that had originated from three-angled fruits and grown in pot 38 along with the three plants just dealt with, gave the following result: 15.32 percent of three-angular fruits, 76.64 percent of four-angular, 6.57 percent of five-angular, 0.73 percent of six-angular fruits, and 0.73 percent of syncarpous fruits.

From a comparison of the figures just given with those in table 9 for the plants in pots 36 and 37, it is evident that the offspring raised from three-angular fruits reproduce the same type and scope of variability as the offspring raised from abnormal, four- or five-angular fruits. The similarity of conditions prevailing in all the three progenies is brought out more plainly by bringing together their respective percentage ratios between the normal and abnormal flowers or fruits (see table 12).

TABLE 12

	No. of plants	Angles of fruits planted	Percentage yields of fruits	
			Normal	Abnormal
Pot 38	3	3	18.60	81.40 (flowers)
	11	3	15.32	84.68 (fruits)
Pot 37	3	4	19.05	80.95 (flowers)
	7	4	17.60	82.40 (fruits)
Pot 36	3	5	17.36	82.64 (flowers)
	7	5	23.02	76.98 (fruits)

From a morphogenetic point of view these results in conjunction with the other similar data collected on the previous generations, are of importance. They indicate that the descendants of this race reproduce the ever-sporting type of the mother plant regardless of whether they

originated from normal or abnormal fruits of the parent. This is in accord with the observations of DE VRIES (1910, p. 526) on fasciated races and other ever-sporting varieties, and of BATESON and MISS PERTZ (1900) on *Veronica Buxbaumii*. The normal three-carpelled flower or the triangular fruit of the ever-sporting variety represents only apparent (phenotypic) reversions to the original, normal type of the species; in reality they carry the potentialities of the ever-sporting character which their offspring manifest in practically the same degree as the progeny raised from the abnormal fruits of the race.

This dimorphism is a typical attribute of this ever-sporting line and as constant as the monomorphic, normal type is characteristic of line No. 22. This is demonstrated by the following experiment. As will be remembered line No. 22 produces under favorable conditions as high as 5 percent of four-angular fruits, the remaining being triangular. As soon as the type of this line had been established it became a matter of interest to find out the behavior of the offspring raised from four-angular fruits borne by plants of this strain. In this experiment four-angular fruits of a fourth-generation plant that had developed 97.28 percent of three-carpelled and 2.72 percent four-carpelled flowers, were planted in the summer of 1918, in pot 69 which was filled with rich, fertilized soil and placed in the greenhouse at the AROOSTOOK FARM. As a check triangular fruits of the same plant were planted in pot 51 under the same conditions of environment. From the four-angular fruits only one plant was raised to maturity which yielded 662, or 97.35 percent, of triangular fruits and 18, or 2.65 percent, of four-angular fruits. The two plants that were raised as a check from triangular fruits of the same mother plant yielded 749 or 97.53 percent of triangular fruits, 18 or 2.34 percent of four-angular, and 1 five-angular fruit. These results clearly show that the four-angular fruits which only rarely occur on plants of line 22 reproduce the normal monomorphic type of that line in a degree almost identical with that produced by the triangular fruits of that race.

We may now turn to the variations of the perigone in the fourth-generation plants. The data dealing with this phenomenon are more exhaustive than those for the first generation. They were recorded for the plants in pots 36, 37 and 39, daily, on the same flowers that were discussed above with respect to variations in the gynoecium. The actual distribution of the flowers with regard to the number of leaves in the perigone and the percentage frequencies for each category of flowers of each plant as well as the percentage frequencies for the total number of flowers are given in table 13.

As in the case of the first generation the number of flowers with a normal five-parted perigone is far in excess of the number of flowers with abnormal perigones. The variation is unilateral, the frequencies decreasing as the number of perigone leaves increases. The ratio between the normal and abnormal perigones in the fourth-generation plants is more in favor of the abnormal perigones than in the first-generation plants, owing, undoubtedly, to the fact that in the former case the observations on the variations of the perigone were complete while in the latter case they have not been recorded for a number of flowers.

In examining the flowers it was noted that the variations in the gynoecium were associated with abnormalities in the perigone. This relation is illustrated in table 14.

TABLE 14

LINE No. 5.—*Actual frequencies showing relation between number of carpels and number of perigone leaves.*

Number of peri- gone leaves	Number of carpels																		Total
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		
5	878	2629	250	33	19	20	13	15	1	9	1	—	—	—	—	—	—	3868	
6	86	934	105	25	1	7	1	—	—	—	—	—	—	—	—	—	—	1159	
7	13	114	60	22	4	—	—	—	—	1	—	—	—	—	—	—	—	214	
8	2	10	8	10	5	15	1	10	1	—	1	1	—	—	—	—	—	64	
9	—	—	1	3	6	1	5	3	1	—	—	—	—	—	—	—	1	21	
10	—	—	—	—	—	4	7	11	1	5	1	1	—	—	—	—	—	30	
11	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1	
12	—	—	—	—	—	—	—	—	1	—	1	—	1	—	—	—	1	4	
Total	979	3687	424	93	35	47	27	41	4	16	3	3	—	—	—	1	1	5362	

In this table are presented the actual frequency distributions in relation to the number of carpels and perigone leaves. This relation is more clearly brought out by the percentage frequencies given in table 15.

From tables 14 and 15 it is evident that the variations in the gynoecium are correlated with the abnormalities in the perigone. The normal, three-carpelled flowers show the highest percentage of normal five-parted perigones. In the same measure as the number of carpels increases, the frequency of normal, five-parted perigones, on the whole, decreases, the normal perigone being substituted by those with a higher number of leaves. Thus, perigones with 9 leaves and above are not associated with three-carpelled or even four-carpelled gynoecia, while gynoecia with a number of carpels above 13 are not found to be associated with five-, six-, or seven-parted perigones.

TABLE 15

LINE No. 5.—Percentage frequencies showing relation between number of carpels and number of perigone leaves.

Number of perigone leaves	Number of carpels														
	3	4	5	6	7	8	9	10	11	12	13	14	18	19	
5	89.68	71.30	58.96	35.48	54.29	42.35	48.15	36.59	25.00	56.25	33.33				
6	8.78	25.33	24.76	26.88	2.85	14.89	3.70								
7	1.33	3.09	14.15	23.66	11.43					6.25					
8	0.20	0.27	1.88	10.75	14.29	31.91	3.70	24.39	25.00		33.33	33.33			
9			0.24	3.23	17.14	2.13	18.52	7.32	25.00					100	
10						8.51	25.93	26.83	25.00	31.25	33.33	33.33			
11								2.44							
12								2.44		6.25		33.33	100		
Total	99.99	99.99	99.99	100.00	100.00	99.99	100.00	100.01	100.00	100.00	99.99	99.99	100	100	

This correlation between the number of carpels and the number of perigone leaves will be recurred to in the discussion of the periodicity in the occurrence of the abnormalities in this race.

The behavior of the fourth generation of line No. 22 may be briefly mentioned here. Triangular fruits from a third-generation plant that had grown in the garden in the preceding summer, were planted in pot No. 46 filled with composted soil and placed in the UNIVERSITY greenhouse along with the plants in pots Nos. 36-39, considered above. Three plants whose flowers were examined, yielded 578 or 97.28 percent three-carpelled and 11 or 2.72 percent four-carpelled flowers. These results show a rather lower degree of variability than in the first generation of this line. Compared, however, with the second and third generation of this race, the fourth generation is appreciably more variable.

Fifth generation

The fifth generation of these two strains was grown in the open garden and in the greenhouse at AROOSTOOK FARM in the summer of 1918. This generation has again borne out the constancy of the type of variability of the two lines under a given set of environmental conditions. The series of plants of line No. 5 grown in the greenhouse yielded data given in table 16.

TABLE 16

	Number of fruit-angles				
	3	4	5	6	7
Percentage frequency	34.58	49.40	15.33	0.51	0.17

The fifth-generation plants of this race grown in the garden made a very vigorous growth, as each plant was allowed ample space, 2 feet square, to stimulate the development of fasciations. The mode of variations in the gynoeceum, however, did not deviate materially from the third generation grown in the open in 1917. The examination of these plants gave the percentage frequencies shown in table 17.

TABLE 17

	Number of fruit-angles					
	3	4	5	6	7	8
Percentage frequency ...	50.78	44.47	4.16	0.37	—	0.21

The fifth-generation plants of line No. 22 in the greenhouse yielded 97.35 percent of three-angled and 2.65 percent of four-angled fruits.

The analysis of the five generations of the two strains of *Fagopyrum tataricum* has brought out the following points. The ever-sporting strain, after isolation, at once displayed the highest degree and fullest scope of its variability reached in the subsequent generations under similar conditions. The ever-sporting character was transmitted to all members of subsequent generations with a striking regularity and uniformity in its degree and scope under a given set of conditions of environment. The extent of the reversion of the ever-sporting character towards the normal specific type as evidenced by the lower proportions of abnormal forms in certain generations, was proved to be controlled by the environment since upon bringing up the subsequent generation under the original conditions, the original degree and range of variability was restored. Selection appeared to have no effect whatever upon the improvement of the ever-sporting variety. The effect of nutrition will be considered in a later section of this paper.

PERIODICITY IN THE MANIFESTATION OF THE CHARACTERS OF THE EVER-SPORTING VARIETY

DE VRIES (1905, p. 361, 1910, p. 323) has established for a number of ever-sporting varieties that the occurrence of their abnormalities is subject to a definite law. According to his observations this law is determined by the individual strength of the different organs of the ever-sporting plant which in turn is determined by external conditions of life, primarily nutrition. "The stronger a bud is the more it is liable to produce anomalies" (DE VRIES 1910, p. 324). This would explain the more frequent occurrence of abnormalities in best nourished regions

on the plant so frequently observed in many instances by DE VRIES. In its application to the plant as a whole this law represents an instance of the fundamental rule that every living organism shows in the course of its life a gradual increase in strength to a maximum after which a decline in vigor follows. DE VRIES sees a rhythmic parallel between this general law of periodicity in the life cycle of a plant and the manifestation of anomalies, the height of the development and differentiation of the plant coinciding with the maximum output of abnormalities. This would explain the periodical occurrence of abnormalities at different periods of the life cycle of the plant, as illustrated by numerous examples cited by DE VRIES. According to these considerations the operation of the law of periodicity can be briefly defined by stating that it manifests itself in two ways: in determining the occurrence of abnormalities in relation to position on the different parts of the plant, and in relation to time in the cycle of the plant life.

Apart from DE VRIES's numerous observations many of which, however, are of a casual nature, the validity of this law has been critically tested in only a few instances. BATESON and PERTZ (1900, p. 82) found that the "chief output of abnormal flowers of *Veronica Buxbaumii* takes place in the earlier part of the flowering season, and especially just before the greatest output of flowers, after which time the percentage of abnormality declines." HERIBERT-NILSSON (1912, p. 112) states that the number of flowers with supernumerary stigmas in *Oenothera Lamarckiana* decreases toward the end of the flowering season. TINE TAMMES (1904) in her study on the occurrence of abnormal leaves in *Trifolium pratense quinquifolium* found that the distribution upon the plant of each of the two anomalies, the lateral and terminal doubling of the leaves, is subject to a definite law according to which the maximum output of leaves laterally doubled occurs on the lower part of the primary axis while the terminally doubled leaves occur more frequently in the vicinity of the inflorescence. But as the absolute number of laterally doubled leaves is far in excess of the absolute output of terminally doubled leaves, TAMMES found that young plants of the "five-leaved" clover show higher proportions of abnormal leaves than the fully grown plants. Here, then, are introduced, for the first time, two abnormal characters on the same plant into the study of the behavior of abnormalities, and we note that the maximum output of each of the abnormalities is confined to different parts of the plant. A further instance of the study on the occurrence of two abnormalities on the same plant is

furnished by LEHMAN (1909) in his account of variations in *Veronica agrestis*. He found for *Veronica Tournefortii* that the time of the maximum production of abnormal calyces does not coincide with the period of the maximum output of abnormal corollas with the doubled anterior petals on the same plant. From this he concludes that nutrition does not determine the periodicity in the occurrence of abnormalities.

The studies of TAMMES and LEHMAN would seem to demonstrate the desirability of subjecting the entire period of occurrence of abnormalities to a statistical examination before any definite picture of the actual type of variation of an ever-sporting variety can be drawn. Furthermore, the exact determination of the distribution of the anomalies upon the different parts of the plant may also have a possible bearing upon the study of the effect of nutrition, or starvation upon the manifestation of abnormal characters. If, for example, the maximum output of abnormal flowers of a certain strain occurs on the most differentiated parts of the plant it is clear that scant nourishment will reduce the volume of differentiation and with it also the number of abnormal flowers. But this reduction cannot be ascribed to unfavorable conditions having a direct effect upon the inherent tendency to vary. It is simply due to the lack of the most favorable region for its expression that this tendency appears weakened.

Frequency distribution of different types of flowers with regard to position on the plant

All these considerations made it appear desirable to study the manner in which the abnormal flowers of this race occur on the different parts of the plant and at different periods of the flowering season. The material for this study comprised the fourth-generation plants grown in the UNIVERSITY greenhouse in the winter of 1918, and was so selected as to include progenies of the same plant but originated from different forms of abnormal fruits. Accordingly, 3 plants in pot 36 raised from five-angled fruits were selected for this purpose. The medium in both these pots consisted of ordinary garden soil. In order to determine any possible effect of high nutrition upon the distribution of flowers, 2 plants raised from four-angled fruits but grown in rich, composted soil, were included in the material. We are already familiar with the total number of flowers and the different categories, of these 8 plants as they have been discussed above as representatives of the fourth generation of the ever-sporting race (see table 9).

The distribution of flowers at different periods of the flowering season and on the different parts of the plants was determined by daily picking and examining the flowers as they appeared on the plants. The flowers were daily recorded according to a scheme that permitted the construction of a mental picture of the whole plant and the tracing of each flower to its original position on the plant after all the flowers had been recorded. A few remarks regarding the essential morphological features of our plants will serve to elucidate this scheme as well as the analysis of the collected data.

Under the conditions prevailing in the greenhouse of the UNIVERSITY at Orono the plants (figures 8 and 9) reached an average height of 22 cm. They developed a main stem or primary axis bearing 7, in a few cases 6, alternate leaves. Only the first three buds in the axils of the first three leaves from below developed secondary branching axes which usually bore 3-4 leaves. The buds in the axils of the first, second and seldom the third leaf on the secondary branch developed two, rarely three tertiary branches. The last, 3rd or 4th, leaf on these always subtended the terminal raceme. The flowers produced in the axils of leaves on the secondary and tertiary branches were borne on short peduncles. With the production of the tertiary branches the branching of the plant was concluded. The upper leaves on the main stem, from the fourth leaf on, did not develop branches, the buds in their axils giving rise only to peduncles bearing racemes. From the third leaf on, the main stem gradually grew weaker, tapering upward and ultimately going over into a slender raceme subtended by the last, usually seventh, leaf. An abnormal feature of the plants of line No. 5 was the production of short, proliferating shoots in the axils of the cotyledons.

In daily recording the flowers the position of each flower was first noted in relation to the leaves ($Phyl$) on the main stem or primary axis, that is to say, whether the flower was located on one of the three secondary branches or one of the raceme-bearing peduncles in the axils of the 4th to the 7th leaves. After this general orientation the position of the flower was further determined in relation to the component parts of the secondary system of branching or to the different regions on the axis of inflorescences of the racemes in the axils of the 4th to 7th leaves. A concrete example may illustrate this method of recording the flowers $Phyl_2R_2l_3$ designated a flower located in the axil of the third leaf (l_3) borne by the right secondary axis (R_2) which had originated in the axil of the second leaf ($Phyl_2$) on the main stem; $Phyl_3L_3r_2l_1$

indicated a flower located in the axil of the first leaf borne by the second right tertiary branch (r_2) that had sprung from the axil of the second leaf of the third left secondary branch (L_3) that had originated in the axil of the third leaf ($Phyl_3$) on the main stem. By the use of this method the original position of each flower on the plant could be determined.

Let us now consider first the frequency distribution of flowers with regard to the position on the plant, i.e., whether any form of flowers is produced with any greater frequency in certain regions of the plant than in others. In order to obtain a general information regarding this question the daily recorded data were first grouped so as to give the actual and relative output of each kind of flowers produced by each organ of the plant, as a whole, originated in the axils of each of the 7 leaves on the main stem. In table 18 are given the actual and percentage frequencies of the flowers for each of the organs sprung from the axillary buds on the main stem. These data represent summarized results taken from tables prepared for each plant separately. The first horizontal row gives the flowers borne in the axils of the cotyledons.

From an examination of this table the following points are evident. The percentage frequencies of the normal three-carpelled flowers show a fairly marked uniformity for each of the different organs of each plant. These frequencies approach very well the percentage frequency of normal flowers in all the 8 plants as a whole, which is 17.98 (see table 9). From the fact that the percentage ratios between the normal and abnormal flowers of each plant organ are markedly uniform it may be concluded that the causes that govern the percentage ratios between the normal and abnormal flowers for the whole plant obtain also with marked regularity in each individual organ of the plant.

With regard to the range of variability or its intensity, as measured by the frequency occurrence of the most aberrant variants we may clearly discern in table 18 that there are regions on the plant that show a wider scope of variability than others. The first two branches, and especially the second one, that yielded the highest output of flowers, mark also the place of greatest variability on the plant as evinced by the great amplitude of aberrant flower forms including the greatest number of synanthous flowers. The synanthous flowers borne by the racemes in the axils of the 4-7 leaves were all contributed solely by the plants in pot 36 which were raised from five-angled fruits. This may indicate a slightly greater intensity in the variability of plants originated from

LINE No. 5.—Actual (f) and percent

Position of flowers	3		4		5		6		7	
	f	percent	f	percent	f	percent	f	percent	f	per
Cotyledons	97	16.33	389	65.71	60	10.14	15	2.53	3	0
Branch 1.....	234	18.75	829	66.43	105	8.41	21	1.68	8	0
Branch 2.....	245	18.39	880	66.07	98	7.36	25	1.88	14	1
Branch 3.....	242	17.60	927	67.42	111	8.00	25	1.82	8	0
Raceme in axil of leaf 4.....	37	16.30	167	73.57	15	6.61	2	0.88	1	0
Raceme in axil of leaf 5.....	20	15.75	93	73.23	11	8.66	1	0.78		
Raceme in axil of leaf 6.....	38	19.10	144	72.36	11	5.93	2	1.01	1	0
Terminal raceme above leaf 7..	66	19.19	258	75.00	13	3.72	2	0.58		
Total	979		3687		424		93		35	

LINE No. 5.—Frequency dis

Position of flower	3	
In axil of l ₁	6	
In axil of l ₂	8	
In axil of l ₃	26	
In axil of l ₄	16	
At base of raceme (bs.).....	35	1
At apex of raceme (ctr. tr.)..	112	30
Total	203	60
Percentage	20.02	65

LINE No. 5.—Frequency distributio

Position of flower	3	4
In axil of l ₁	27	78
In axil of l ₂	16	77
At base of raceme (bs.).....	22	85
At apex of raceme (ctr. tr.)..	61	192
Total	126	432
Percentage	18.72	64.19

five-angled fruits than in those from three- or four-angled fruits, a condition not infrequently observed in this race.

From table 18 it is further to be noted that the range of variability shown by the flowers borne by the racemes in the axils of the 4-7 leaves is very limited as is also the actual number of flowers. From the totals in the last vertical row in table 18 we note the yielding capacity of each plant organ. The three branches in the axils of the three lowest leaves yielded the highest output of flowers; the cotyledonary organs range next, to be followed by the terminal raceme and the racemes in the axils of the 4th leaf on the main stem; the racemes in the axils of the fifth and sixth leaves yielded the lowest number of flowers. From these data we may conclude that the yield of Tartary buckwheat plants is determined, broadly speaking, by the basal regions of the plant. This fact is of importance in relation to the aims of practical breeding, and suggests the desirability of adopting such cultural methods as would favor the development and differentiation of the lower plant body rather than the extension of the upper part at the expense of basal differentiation.

Table 18 gives the general distribution of flowers upon the different plant organs taken as a whole. We may now analyze these organs into their differentiated components. Four plants, No. 5, 6, 7 and 8, were selected for this analysis. As already noted, only the three lowest axillary buds on the main stem gave rise to differentiated branching organs, each producing 2-3 tertiary branches. In table 19 is given the frequency distribution of flowers upon the secondary branches alone, excluding the tertiary ones. The flowers are grouped here in six categories. In the first four classes are grouped the flowers that were borne in the axils of the 1st-4th leaves (l_1, l_2, l_3, l_4) on each of the secondary branches. The last two categories include flowers borne exclusively by the terminal raceme of each secondary branch. On these terminal racemes the flowers were recorded for three regions of the axis of inflorescence: The flowers in the basal region of the axis of inflorescence (bs.) the terminal flowers (tr.) and those immediately below and close to them (ctr.). The flowers recorded for the last two regions are grouped in one class in table 19 (ctr., tr.).

In table 20 is presented the frequency distribution of flowers upon the tertiary branches.

Both tables 19 and 20 represent summarized results taken from tables made out for each plant separately. A comparison of the data given in both tables shows that the distribution of flowers upon the

secondary and tertiary branches have certain points in common, though an absolute comparison is not permissible since the tertiary branches did not develop the third and fourth leaf. From the totals for each component of the secondary and tertiary branches it will be noted that the highest output of flowers was produced in the apical region of the terminal raceme and the next highest in the basal zone of the axis of inflorescence. If the production of synanthous flowers is indicative of a high tendency to vary and proliferate it would point to the basal region of the terminal raceme as the seat of greatest variability. This is brought out by tabulating the percentage frequencies of synanthous flowers for each organ of each branch system as illustrated in table 21.

TABLE 21
Synanthous flowers in percent.

Position of flower	Secondary branch	Tertiary branch
1 ₁		2.44
1 ₂	1.33	3.33
1 ₃	0.71	
1 ₄		
bs.	5.16	3.45
ctr. tr.	1.26	1.75

From tables 19 and 20 it will further be noted that while the percentage ratio between the total number of normal and abnormal flowers of the secondary and tertiary branch, respectively, very well agrees with that for the plants as a whole, the percentage ratio between the normal and abnormal flowers for each individual organ of both branching systems shows a considerable deviation from the average percentage for the whole branching system. In spite of the relatively small number of flowers involved these deviations are undoubtedly significant since the corresponding homologous component elements of the secondary and tertiary systems show an analogous rise and drop in the proportion of normal flowers. This may be seen from the last vertical columns in tables 19 and 20. Thus the axillary buds of the first leaves favor the production of normal flowers, 25 percent and 21.95 percent, respectively; the buds in the axils of the second leaves appear to produce the lowest percentage of normal flowers, 10.53 percent on the secondary branch and 13.33 percent on the tertiary branch. The axillary buds of the 3rd and 4th leaves on the secondary branch produce the average proportion of normal flowers, while at the base of the terminal racemes the propor-

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LINE No. 5.—Percentage frequencies of 7 days.

Date							Age ratio between normal and abnormal flowers
	3	4	5	6	7	8	
March 6-12.....	27.85	54.38	13.38	1.86	0.27	0.27	85 : 72.15
March 13-19.....	23.17	67.56	5.85	0.73	0.49	0.23	17 : 76.83
March 20-26.....	15.06	72.81	5.17	2.25	0.89	0.15	06 : 84.94
March 27-April 2	14.56	66.84	7.72	3.68	0.88	1.44	56 : 85.44
April 3-9.....	19.31	65.15	8.95	1.26	0.78	0.10	31 : 80.69
April 10-16.....	11.74	71.68	8.12	2.07	0.52	1.11	74 : 88.26
April 17-23.....	18.65	70.68	4.81	0.90	0.60	0.18	65 : 81.35
April 24-28.....	20.72	68.63	6.27	0.76	0.76	20.72	: 79.28

LINE No. 5.—Percentage frequencies of 6 periods

Date			Age ratio between normal and abnormal flowers
	5	6	
March 6-12.....	81.96	14.59	
March 13-19.....	80.00	15.12	
March 20-26.....	60.67	28.09	
March 27-April 2	72.63	19.65	
April 3-9.....	71.90	19.62	
April 10-16.....	71.50	21.93	
April 17-23.....	63.46	28.87	
April 24-28.....	64.64	28.71	

tion of normal flowers drops to finally increase again above the average in the apical region of the racemes.

From the data presented in tables 18-20 the following conclusions may be drawn with reference to the distribution of flowers upon the plants of this race. The greatest output of flowers was produced in the lower, differentiated parts of the plant. The ratio between the normal and abnormal flowers appears fairly uniform under the prevailing conditions of environment, for each of the collective plant organs arisen from the axillary buds on the main stem. The analysis of the collective organs into their component parts reveals a marked variation in the percentage frequencies of normal and abnormal flowers produced by the axillary and terminal buds on the secondary and tertiary branches, the axillary buds of the second leaf and the basal zone of the terminal raceme producing the highest relative number of abnormal flowers. Relative to the range or intensity of variability it is again the lower, most differentiated parts of the plants, more specifically the component parts of the second and third secondary branch, especially the former, that exhibit the widest amplitude of aberrant flower forms. To specify it more closely, the basal regions of the terminal raceme of the secondary and tertiary branches are the seat of the greatest tendency to proliferate as evinced by the wide range of aberrant forms. This is in accord with the observations of DE VRIES (1910, p. 329) who found the lower end of many racemose inflorescences to be a place specially favorable to anomalies.

FREQUENCY OF OCCURRENCE OF DIFFERENT TYPES OF FLOWERS IN RELATION TO DIFFERENT PERIODS OF THE FLOWERING SEASON

Having considered the distribution of flowers with reference to their position on the plant body, we may next inquire into the frequency of occurrence of the different flowers in relation to the different periods of the flowering season. For this purpose the daily recorded data were brought together into 8 groups, each (except the last one) covering a successive flowering period of 7 days, and all making up the entire flowering season during which 5444 flowers were recorded.

In table 22 are given the percentage frequencies of the flowers with respect to number of carpels computed on the basis of actual frequencies noted for each successive week.

From the data presented in table 22, it is evident that the first two weeks of the flowering season mark the lowest relative production of abnormal flowers. From the second week the relative total number of

abnormal flowers increases to reach a maximum in the third and fourth week. In the course of the fifth week, April 3-9, the proportion of abnormal flowers drops markedly to reach another more distinct maximum in the sixth week. The last two weeks of the flowering season again mark a slow decrease in the relative production of abnormal flowers.

The rather striking depression in the relative production of abnormalities in the gynoeceium in the course of the fifth week is of interest. That this depression is significant is shown by the figures given in table 23, which indicate that at about the same period a similar reduction occurred in the relative output of abnormalities in the perigone, which, as will be recollected (see table 15) are associated with abnormal gynoecea.

From table 23 it will be noted that also in the case of the perigone variations the first two weeks mark the lowest relative production of abnormal perigones. The maximum was reached in the course of the third week, after which, in close analogy to the variations in the gynoeceium, a depression followed, which, however, in this case extended over a period of three weeks when the abnormalities again increased..

The similarity of effect upon both kinds of abnormalities would point to a common cause which in the case of the perigone variations was more effective than in the variations of the gynoeceium. The nature of this cause may have been internal or external. About the fourth week of the flowering season, the plants, owing to the continuous removal of their flowers, gave rise to abnormal growth by producing several short shoots in the axils of the cotyledons bearing numerous flowers. It is conceivable that this abnormal vegetative growth may have caused a disturbance in the function of the specific determiners conditioning the manifestation of abnormal flowers. A more plausible explanation, however, may be found in external influences of environment. The plants of the race used in this investigation grew along with a host of other plants that were frequently watered; this caused the air to be very moist and damp. During the first week of April, owing to the illness of the man in charge of the greenhouse, the plants were not watered frequently, with the result that the air in the greenhouse during that period was very dry. The concurrence of this period of dryness with the reduced output of abnormal flowers would point to moisture as the factor of environment facilitating the realization of the abnormal flowers in this race. This was also borne out by results obtained from cultures grown under different conditions of environment, though further and special experiments are yet required to obtain conclusive evidence on this question.

Neglecting for a moment the above disturbance in the floral variations we may conclude from an inspection of the data given in the last vertical rows in tables 22 and 23, that under the prevailing conditions the first and second week of the flowering season mark the lowest relative production of abnormal flowers, after which a marked and sudden increase in the output of abnormalities ensues. To that extent our data are in accord with the observations of DE VRIES. But the behavior towards the end of the flowering season, which is marked by only a small decrease in the percentage of abnormalities, does not conform to the rule established by DE VRIES, according to which the relative output of abnormality shows a rapid and marked decline towards the final period of the flowering season. This behavior of our race was found to be due to the manner in which different flowers are distributed upon the different parts of the plant. As it will be recollected (see table 18) the racemes borne in the axils of the 4th-7th leaves show the narrowest range of variability. Now, it is just this section of the plant, from the fourth leaf up, that contributes the bulk of flowers during the first two weeks of the flowering season. During the following weeks the differentiated organs of the lower section of the plants, the secondary and tertiary branches, come into play, raising very markedly the percentage of abnormal flowers since these differentiated branches, as will be remembered, are the very seat of greatest intensity of variation. Towards the end of the flowering season the upper regions of the plants produce only very few flowers while the lower-differentiated parts sustain their flower production to the end of the flowering season. This differential distribution of flowers upon the parts of the plant and the sequence in the unfolding of the flowers explains the relationship, referred to above, between the normal and abnormal flowers during the different periods of the flowering season: the plants begin flowering in the region marked by a narrow range of variability and finish in the regions of greatest intensity of abnormal development.

In considering the periodicity in the distribution of normal and abnormal flowers of this race it should be borne in mind that the plants used in this investigation were grown in the greenhouse and under conditions manifestly favoring the development of abnormalities. It is quite possible that the conditions prevailing in the open might operate in a different manner upon the manifestation of abnormality than in the greenhouse. With this reservation in mind, we may state that the examination of the distribution of the flowers upon the different parts of

the individual plant points, in conformity with the observations of DE VRIES and other writers, to the existence of a specific region on the plant in which the tendency to vary and to proliferate is most pronounced. Contingent upon this distribution with respect to position on the plant, and, indeed, a function of it, is—barring external interference—the varying percentage ratio between the normal and abnormal flowers in the successive periods of the flowering season.

FASCIATION IN *Fagopyrum tataricum*

A further teratological feature exhibited by this race is fasciation. The writer is aware of no previous record of fasciation in *Fagopyrum tataricum*.

Fasciation was first observed on plants of the fourth generation grown in the UNIVERSITY greenhouse in 1918, the variations of which have been the subject of the discussion in the preceding section. Fasciated branches were found on plant 8 growing in pot No. 36 and on plant 6 and 5 in pots 37 and 39 respectively. It should be stated that three of these four plants have not been included in the study of periodicity and, consequently, were not subjected to any possible traumatic influence entailed by the daily removal of flowers.

The first indication of a fasciated condition was noted in the altered phyllotaxy. The leaves at the first node on the main axis were inserted opposite each other instead of the normal alternate insertion. The branch that sprung from their axillary bud presented a flattened ribbon-like condition with a distinct groove running up to the summit at which point the branch resolved itself into two subdivisions each bearing a raceme. As the plants were small (about 25 cm tall) and fairly crowded in the pots the fasciations were not very conspicuous. It was not until the summer of 1918 that beautiful specimens of fasciations were obtained among the cultures grown in the garden. In order to stimulate the development of fasciations by abundant nutrition, the fruits from plants in the pots Nos. 36-39 as well as from some other plants of this race were planted out 2 feet apart each way. The plants made a very vigorous and luxuriant growth developing strong primary and secondary branches. It was the latter system of branches and among these usually the lower ones, at the first node above the cotyledons, that presented the best specimens of fasciation.

The morphological aspect of these fasciations was generally similar to that recorded in other genera. The fasciated branches often presented

below a nearly cylindrical form, which in the upper part changed into a flattened condition, and ultimately, at one of the nodes split into two or more branches of more or less normal aspect. Such a case is illustrated in figure 5. From this figure the altered arrangements of the



FIGURE 5.—Fasciation in *Fagopyrum tataricum*. A fasciated branch showing altered phyllotaxy and splitting of branch at node into 2 subdivisions. Nat. size.

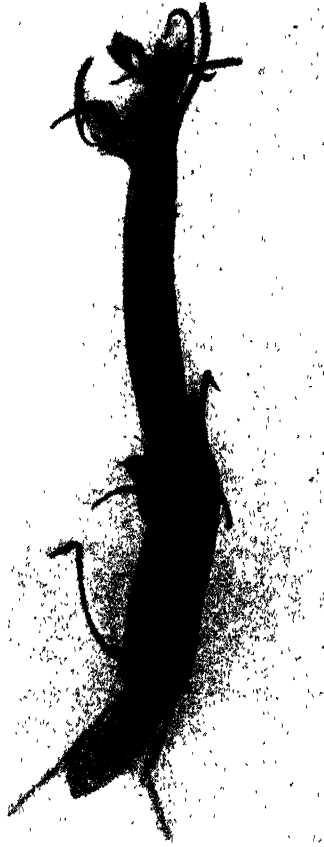


FIGURE 6.—Fasciation in *Fagopyrum tataricum*. A highly fasciated branch displaying a flattened ribbon-shaped condition throughout its entire length. Nat. size.

leaves and branches can be clearly seen, which are numerous and whorl-like crowded around the nodes. Quite often branches were found to be flattened in distinctly ribbon-like fashion throughout their length, in which case they would rarely split but continue in that condition narrowing gradually towards the summit where they would resolve themselves into numerous proliferating shoots, leaves and flowers (figure 6); often fasciated branches of this type were bent, owing, undoubtedly, to unequal growth.

A rather interesting fasciated branch was observed on a fifth-generation plant that grew in pot 64 in the greenhouse at the AROOSTOOK FARM, and had originated from a four-angled fruit of plant 5, from pot 37 of

the fourth generation. A rough diagrammatic sketch of that branch is given in figure 7. This branch originated in the axil of the first leaf above the cotyledons. It was 1.2 mm thick and 4 mm wide for a distance of 21 cm. At this point the branch twisted itself to the right and split in the middle: the two separated parts enclosing a more or less triangular space. This split was probably caused by tissue tension in the knee-shaped bend. The upper separated part of the branch measured 2 mm, the lower 2.5 mm in width. Just above the point where the branch resumed its undivided aspect again, it measured 5.5 mm. From that point the branch turned upwards twisting round its axis from right to left for a distance of about 1.5 cm when it ran almost horizontally twisting again round its axis. The width measured here was 5 mm. At this point the branch turned inward measuring 4 mm in width and terminating, perfectly ribbon-shaped, in a cluster of fruits. The leaves, with the exception of the first one, showed altered phyllotaxy. Four pairs of leaves developed bearing in their axils fruits on short peduncles. At the point where the branch took a nearly horizontal course a pair of rudimentary leaves developed with a cluster of fruits distributed around the node. At the apex of the branch the largest cluster of fruits was produced with no trace of leaves.

With regard to the transmission of the fasciated character our race exhibited a dimorphism characteristic of the ever-sporting varieties. The figures in table 24 illustrate the behavior of the fasciated character in plants of the fifth generation that grew under favorable conditions of nutrition.

TABLE 24

Source of fruits	Number of plants	Number of fasciated plants
Pot 36	25	7
Pot 37	11	4
Pot 38	8	5
Pot 39	27	15
Pot 42	16	10
Pot 44	11	5
Pot 47	8	5
Total	106	51

The fasciation thus was reproduced in half of the progeny. Pending the further behavior of the fasciated character of this strain in subsequent generations and its analysis by crossing, the question of its heredi-

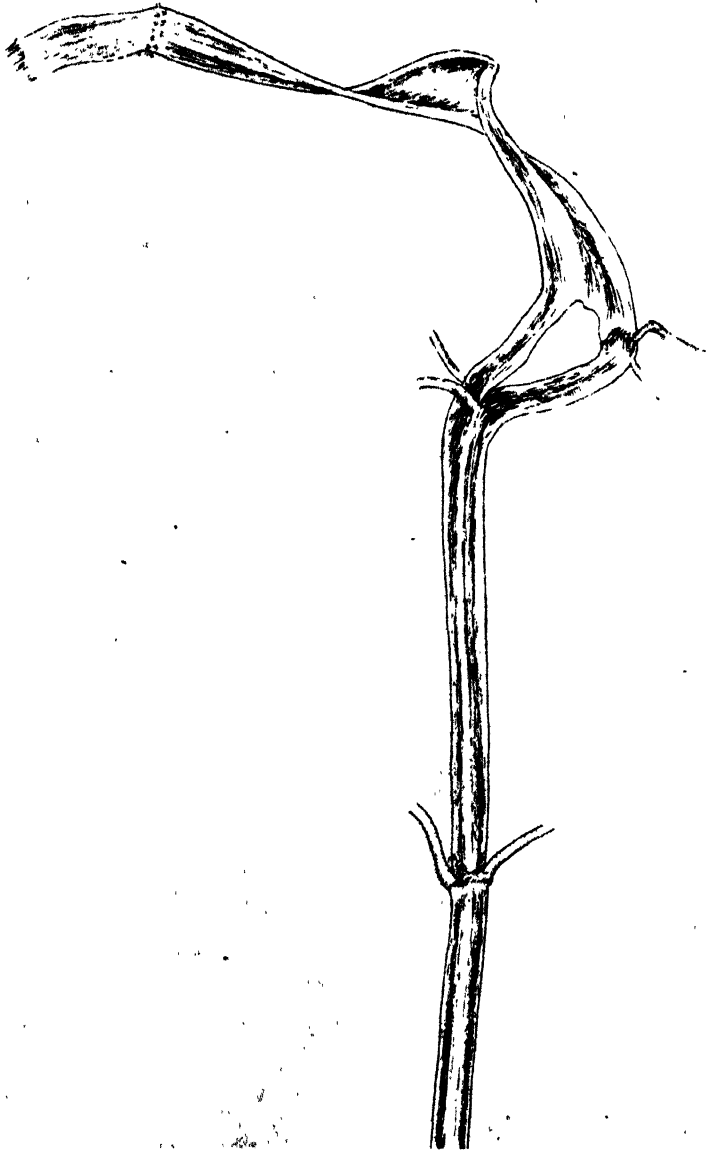


FIGURE 7.—Fasciation in *Fagopyrum tataricum*. Diagrammatic sketch illustrating a peculiar case of fasciation.

tary transmission cannot yet be definitely answered. The manifestation of this character was first noted in plants that grew under crowded

conditions in pots. In the following generation, under favorable conditions of nutrition, this character at once asserted itself in a manner typical of the fasciated races. It is important to state here that the slightly varying line No. 22, though grown under the same conditions of environment as line No. 5, was never found to exhibit fasciation even in a single individual. All external agencies, as traumatic stimuli, insects, etc., had the same chance of operating upon the individuals of both lines in the direction of evoking fasciation, as the progeny of both lines grew side by side, and in some instances, in the same pots. We must note then the fact that, whatever the nature of this fasciation, the two strains react in a different manner to the influence of the same environmental agencies, as evinced by their different phenotypic appearance.

Notwithstanding these considerations it is realized that it is premature to commit oneself on the question of heritability of this fasciation. It would be equally premature to *a priori* deny the hereditary character of the fasciation in question as some writers do with most of the fasciated races of DE VRIES which they dismiss as mere somatic modifications without previously furnishing the necessary experimental evidence from a genetical analysis of the cases. The fact that certain experimenters succeeded in evoking fasciations through artificial stimuli or operation of parasites does not necessarily disprove the interpretation of most of DE VRIES's fasciations as hereditary characters. It is well known that most of the fasciated races exhibit a "fixed dimorphism," one part of the progeny displaying the character of fasciation and the other part appearing perfectly normal. This latter group, however, is only apparently normal for in the next generation they reproduce, under similar conditions of environment, the same proportion of normal and fasciated plants as the members of the fasciated group, much in the same fashion as the fruits of a short-styled plant of the heterostylous *Fagopyrum esculentum* produce short-styled as well as long-styled plants in about equal numbers. It is clear then that it is incumbent upon those who from instances of artificially evoked fasciations draw conclusions disproving the hereditary character of most of the fasciations, to prove that the material that reacted to external stimuli with the formation of fasciation did not belong to the group of phenotypically normal plants but with a genotypic condition for fasciation originally present.

External agencies may well induce fasciations operating as a *releasing* agency in cases where the factor for fasciation is present, much in the same fashion as temperature causes chemical reactions to take place;

but there seems to be no available evidence showing the external agencies to be the direct *causal* factor in the etiology of fasciations. The fact that artificially induced fasciations have been established in the very plants (*Zea Mays*, *Oenothera*, *Raphanus raphanistrum*, *Picris hieracioides*) which without provocation by external injurious agencies, spontaneously develop fine specimens of fasciation, very strongly suggests that the fasciations following upon external injuries are a secondary phenomenon contingent upon a genotypic condition for fasciation already present. In this connection it is of interest to note that PEYRITCH (cited by GOEBEL 1900, part I, p. 188) whose researches into the teratological development as induced by external injurious agencies, are well known, clearly distinguished between "the immediate *determining* cause, which in many cases may be an external agent, and the internal factor, namely the *predisposition* to the development of the anomaly. It is easy to convince oneself that all the individuals of the same species do not react in the same way towards the same external injurious agencies, and that their reaction also varies at different times."

OBSERVATIONS ON THE INFLUENCE OF THE ENVIRONMENT UPON
TERATOLOGICAL DEVELOPMENT IN *Fagopyrum tataricum*

In dealing with the behavior of ever-sporting varieties DE VRIES (1910, p. 307) emphasizes with great stress the dependence of their "semi-latent" characters upon the external conditions of life. His observations and experiments led him to the conclusion that "increased nutrition favors the development of the anomaly."

BAUR (1907) formulated a theory based chiefly upon the observations of DE VRIES, and designed to explain the behavior of ever-sporting varieties like *Dipsacus silvestris torsus* or even *Matthiola* as specific instances of modifications determined by external factors, nutritional factors in the broadest sense.

In view of the great importance attached to nutrition as highly influencing or even directly determining the characters of the ever-sporting races, it seemed desirable to test the influence of nutrition upon the race dealt with in this paper.

In the experiments to be now described the behavior of this race was studied in different nutritional media and in different environments. The media used were composted soil, ordinary soil and sand. As to environment, the cultures were grown under greenhouse conditions and in the garden. Two greenhouses were used whose conditions differed greatly,

notably with respect to humidity and temperature. In the greenhouse of the UNIVERSITY prevailed what might be called a moist and hot condition. The temperature was uniform, varying only slightly from 24° C during the day to 21° C at night. The greenhouse at the AROOSTOOK FARM where the cultures grew in the summer, no artificial heat was used, the temperature following the natural daily amplitude. The air in this greenhouse was dry.

The effect of deficient nutrition or starvation was studied through five generations and in each generation from the second on the plants grown in sand or gravel originated from fruits of plants that also had grown under conditions of starvation. Thus subjecting a part of our cultures to the influence of starvation from generation to generation a pure sand strain has been obtained which offered the opportunity of studying not only the effect of the immediate starvation medium but also the influence of a continued operation of deficient nutrition affecting the plants of each generation in their ontogeny, during the critical period of seed formation, in the course of which, according to the observations of DE VRIES and of EAST and HAYES (1914, pp. 35 and 47) certain plant characters are susceptible to the operation of environmental agencies.

A portion of the fruits of the original plant from which line No. 5 descended was planted in an 8-inch pot No. 2 filled with soil that was mixed with commercial fertilizer. The other portion of the same crop was planted in pot No. 2a filled with soil that was mixed with a considerable amount of sand. Both pots were placed in the UNIVERSITY greenhouse.

The appearance of the sand cultures did not differ very markedly from those in the fertilized soil. The detailed account relating to the variation of the plants in these 2 pots is given in tables 1 and 3, respectively. From the almost identical manifestation of abnormality in both series it may be concluded that the poor medium in pot No. 2a had no limiting effect in the development of abnormal blossoms.

The fruits from pot No. 2 were planted in the summer of 1916, in pots filled with good soil mixed with fertilizer, while the fruits from pot No. 2a were planted in pots containing gravelly sand. The pots of both series were placed side by side in the garden. The ratios between the normal and abnormal flowers in both series are given in table 25.

The plants in the rich soil yielded twice as many fruits as those in the sand but as will be seen from the above figures, the mode of variation in the gynoeceum is strikingly similar in both series.

TABLE 25

	Fertilized-soil series				Sand series			
Number of carpels.....	3	4	5	6	3	4	5	6
Actual frequency	864	396	34	1	486	233	10	1
Percentage frequency ...	66.71	30.58	2.63	0.07	66.57	31.92	1.37	0.13

In the following season the plants of each of the two series were subjected to the treatment accorded their respective mother plants. The pots of both series were again placed out-doors. The plants in the rich soil made a tall growth with a relatively moderate amount of branching in the lower parts of the plant body, while the sand cultures were of a low stature. The results from both series are shown in table 26.

TABLE 26

	Rich-soil series				Sand series			
Number of carpels.....	3	4	5	6	3	4	5	6
Actual frequency	1366	1000	112	4	520	559	68	2
Percentage frequency ...	55.04	40.29	4.51	0.16	45.26	48.65	5.95	0.17

The plants in the sand series actually yielded a higher percentage of abnormal flowers than those in the rich soil.

The behavior of the two series under their respective treatment was again studied in the fourth generation grown in the UNIVERSITY greenhouse. The progeny of the plants of the rich-soil series were grown in ordinary and composted soil, in pots 36-39; the progeny of the plants in the sand series were grown in sand, in pots 43 and 44. The morphological features of the plants in 36-39 will be recalled from the discussion given in a previous section. They made a compact growth marked by differentiation and branching in the lower part of the plants (figures 8 and 9).

The plants in pots 43 and 44 that had originated from fruits of "starved" parents presented a rather interesting aspect. They were of very low stature, dwarf-like in appearance, reaching a height of only 6 to 12 centimeters (see figure 10). The leaves of these plants were small and their number slightly lower than in the plants originated from well nourished parents (cf. EAST and HAYES 1914). The size of the corollas and of the fruits was normal.

The examination of the flowers of the plants grown in composted soil,

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TABLE 27

Medium	Number of carpels																Synanthies
	3	4	5	6	7	8	9	10	11	12	13	14	16	17	18	19	
Composted soil ..	16.28	67.14	8.64	2.11	0.82	0.74	0.52	1.08	0.14	0.71	0.17	0.06			0.06	0.06	1.49
Ordinary soil	19.50	67.05	7.73	1.33	0.66	0.88	0.57	0.55		0.09			0.04				1.50
and	17.36	61.43	13.61	1.95	0.51	0.41	0.10	0.41	0.10	0.10		0.10		0.10			3.81



FIGURE 8.—Greenhouse cultures of *Fagopyrum tataricum*. A number of plants of the ever-sporting line No. 5, grown in ordinary garden soil. $\times .4$.

normal soil, and sand, gave the results in table 27, expressed in percent.

These figures bear out the results obtained from the first generation



FIGURE 9.—Greenhouse cultures of *Fagopyrum tataricum*. Plants of line No. 22 grown in composted soil. $\times .4$.

indicating that under the conditions prevailing in this greenhouse rich nutrition or starvation has no visible effect upon the development of ab-



FIGURE 10.—Greenhouse cultures of *Fagopyrum tataricum*. Dwarfed plants of line No. 5 grown in sand. $\times .4$.

normal flowers. Starvation had a marked effect on the habit of the plants affecting such characters as height and number of leaves.

This experiment was again carried out with cultures of the fifth generation. The plants of the sand series were grown in pots and placed in the garden. The plants originated from the well nourished parents were grown in the garden under very favorable conditions of nutrition, each plant occupying 2 feet each way. The sand series yielded 53.13 percent of normal and 46.87 percent of abnormal fruits. The garden plants yielded 50.78 percent of normal and 49.22 percent of abnormal fruits.

From the evidence furnished by the above experiments the conclusion may be drawn that under conditions favoring the maximum degree of abnormal development optimum nutrition or starvation has no visible effect upon the degree of manifestation of floral abnormalities in the race dealt with in this paper. With the elimination of nutrition as a stimulating factor of environment, all the evidence obtained from the behavior of this race under different environments, notably the identical mode of variation of the first and fourth generation grown in hot and moist air, points to high humidity and temperature as the factors



FIGURE 11.—Greenhouse cultures of *Fagopyrum tataricum* Plants of line No. 5 grown in sand and scantily watered. $\times .4$.

favoring the maximum expression of abnormal development.² Instances showing the development of abnormalities to be dependent upon factors of environment other than nutrition, are by no means rare. Thus a mutation in *Drosophila*, the reduplication in the legs, discovered by Miss HOGE (1915), was found to be dependent upon low temperature for its realization. Another mutation in the same fly, "abnormal abdomen," reported by MORGAN (1915), was shown to be determined by the amount of moisture in the food.

Where the conditions of environment, necessary for the realization

² It is conceivable that the reduced volume and intensity of the light in the early spring months as compared with that of the summer months, may have influenced the teratological development in the first and fourth generation of this race. However, neither the habit of the plants, nor the daily recorded mode of variation seemed to support this assumption.

of the maximum of abnormal development are not present in the required degree, as in the cool and dry greenhouse, starvation and other detrimental agencies may assert their influence upon the development of abnormality. Some evidence supporting this point may be gathered from the following experiment. In connection with the growing of the fourth generation of this race, an attempt was made to determine the combined effect of starvation and lack of water under greenhouse conditions. Accordingly, some 26 plants that had originated from well nourished parents were grown in an 8-inch pot filled with sand and placed in the UNIVERSITY greenhouse in which hot and moist air prevailed. The plants were watered only very scantily,—barely enough to keep them from drying up. As a result the plants were stunted (figure 11) with thin stems and hardly any secondary branches. The extent of the stunting in growth can be seen by comparing the measurements of the laminae of both cotyledons of these plants with those of the normal plants grown in rich soil and well watered. Both cotyledons of each plant were measured, 2 diameters of each lamina being taken: the diameter in the plane of the stalk (d_1) and one at a right angle to it (d_2). The measurements were recorded for 10 plants in each series. The average length, with the normal plants, of d_1 was 17.05 mm and of d_2 19.75 mm, while the same dimensions with the plants of the sand series were 10.86 mm and 12.42 mm, respectively. The examination of the flowers of 8 plants grown in sand and scantily watered gave the results tabulated in table 28.

TABLE 28

	Number of carpels						Synanthies
	3	4	5	6	7	8	
Actual frequency	47	223	16	2	1	1	3
Percentage frequency ...	16.03	76.11	5.46	0.68	0.34	0.34	1.03

From these figures it will be noted that the ratio between the normal and abnormal flowers, 16.03:83.97, of this series is practically the same as in the well watered cultures grown in composted soil (16.28:83.72).

The same experiment was carried out in the cool and dry greenhouse at AROOSTOOK FARM. The results obtained from the normal, well nourished and watered cultures, from normally watered sand-cultures and from scantily watered sand-cultures are given in table 29.

TABL F. 29

Medium	Treatment	Percentage ratio between normal and abnormal flowers
Rich soil	Well watered	34.58 : 65.42
Sand	Well watered	46.36 : 53.64
Sand	Scantily watered	68.40 : 31.60

These figures in conjunction with the results obtained from the plants grown in hot and moist air clearly indicate that under conditions void of optimum moisture and temperature necessary for the maximum development of abnormality, the influence of deficient nutrition and lack of water becomes apparent but that the effect of these detrimental factors is nil when they operate in the presence of other factors of environment which control the maximum manifestation of the abnormal character.

SUMMARY

The more important observations recorded in this paper may be summarized as follows.

An ever-sporting race of *Fagopyrum tataricum* has been isolated and its characters studied for 5 generations under varying conditions of environment.

The variations here considered occur in the gynoecium, the perigone, and the vegetative organs of this race.

The variations in the gynoecium are characterized by the production of supernumerary carpels. The number of carpels per pistil was found to vary from 3 up as high as 25. Under ordinary conditions of growth the number of flowers with normal gynoecia is greater than or equal to the number of flowers with abnormal gynoecia. Under conditions favoring the development of abnormal flowers the variation is bilateral, and can be represented by a curve the apex of which is formed by the abnormal four-carpelled flowers.

The frequency of flowers with abnormal gynoecium decreases as the number of aberrant carpels per pistil increases.

Associated with the abnormal gynoecia are abnormal perigones with a varying number of segments ranging from the normal number 5 as high as 18. The favorable conditions capable of transforming the unilateral variation of the gynoecia into a bilateral one, failed to affect the perigone in the same manner. The variation in the number of perigone leaves remained unilateral with the frequency of the normal, five-parted perigone forming the apex of the skew curve.

The frequency of the normal, five-parted perigone decreases as the number of carpels per pistil increases.

Floral proliferations in the form of various types of synanthous flowers, often giving rise to syncarpous fruits, were found to be produced generation after generation in fairly constant proportions under given conditions of environment.

The teratological development of the vegetative organs in the form of more or less developed fasciations was reproduced, under favorable conditions of environment, in 50 percent of the offspring.

All the descendants of the ever-sporting race reproduce the ever-sporting type of the mother plant regardless of whether they originated from normal or abnormal fruits of the parent.

The ratio between the normal and abnormal flowers was found to be a function of the environment. Under a given set of environmental conditions this ratio as well as the relationship between the different forms of the abnormal flowers *inter se* is constant to a very marked degree.

Selection carried on for 5 years had no visible effect upon the type and range of teratological development of this race. The ever-sporting strain after isolation at once displayed the highest degree of abnormality reached in the subsequent generations under similar conditions of environment.

Under conditions controlling the intensity of abnormal development, optimum nutrition or starvation, while affecting the habit of the plants, appeared to have no effect upon the degree of manifestation of abnormalities. The evidence from the study of this race under different conditions of environment points to high humidity and temperature as the factors favoring the expression of abnormality. Under unfavorable conditions of humidity and temperature, the influence of starvation and lack of water upon the degree of abnormal development was noted.

The results of a study of the frequency distribution of the different types of flowers upon the plant point to the existence of a definite region on the plant in which the tendency to vary and proliferate is most pronounced. Considering the plant as a whole, this region is confined to the basal, differentiated parts of the plant. The first three branches on the main stem from below, especially the second one, mark the seat of greatest abnormal development, while the 4th, 5th, and 6th branches show a low degree of variability as well as the lowest absolute number of flowers. In the basal region of the terminal raceme the output of flow-

ers and the range of abnormality again increases. Similar but more marked differences prevail in the individual branches of the second and third order. Here, it is again the buds in the axils of the second leaf and in the basal region of the terminal raceme that show the greatest relative number of abnormal flowers as well as the greatest range of variability as measured by the frequency occurrence of the most aberrant variants.

Relative to the frequency of occurrence of the different types of flowers at different periods of the flowering season, under the prevailing conditions, the first and second week of the flowering season mark the lowest relative production of abnormal flowers, after which a marked increase in the output of abnormalities follows when the secondary and tertiary branches begin to develop their flowers. Toward the end of the flowering season the upper regions of the plants produced only a very few flowers while the lower differentiated parts of the plants sustained their flower production to the end of the flowering season.

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AN HEREDITARY COMPLEX IN THE DOMESTIC FOWL

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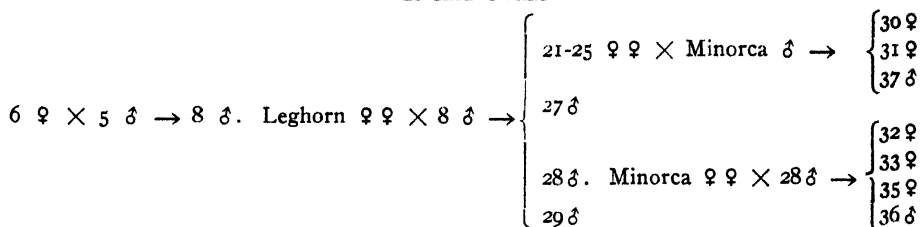
Brachydactyly, syndactyly, and leg-feathering or ptilopody¹ are among the many characteristics of the domestic fowl that have been studied from the standpoint of genetics. The last in particular, owing to its widespread occurrence, has aroused especial interest. Its heredity has been investigated by HURST (1905), DAVENPORT (1909), and more recently by PUNNETT and BAILEY (1918). The genetic behavior of syndactyly has been studied by DAVENPORT (1909) and that of brachydactyly by DANFORTH (1919 a).

The purpose of this paper is to present evidence that these three characteristics, at first sight apparently distinct and unrelated, are in reality the product of a single gene or a single combination of genes. The possibility that this is the case was suggested in the earlier paper on brachydactyly, but at the time that paper was written, sufficiently critical breeding tests had not been made, the suggestion being based largely on morphological evidence. The additional data now available, while not abundant, seem to be significant, and are therefore put on record.

Except where otherwise indicated the descriptions and data are all from individuals derived, on one side at least, from a single family, the relationships of the principal members of which are shown in the accompanying pedigree chart. None of those indicated in the chart was syndactyl.

¹Two terms, "leg-feathering" and "booting" have been employed to indicate the presence of feathers on the tarsus and toes. Both of these terms are inappropriate; the former because it is not the leg, but the tarsus and toes that is meant, the latter because the term is already in use to indicate a fusion of tarsal scutella. Since no other convenient term seems to be available the word ptilopody (πτίλον, feather; πούς, foot) is used in this paper to designate the condition in which down or feathers tend to appear on the tarsus and toes.

PEDIGREE CHART



The individuals of mixed ancestry may be characterized as follows:

No. 5. Male. A mongrel of unknown ancestry, but with a general appearance suggestive of Brahma extraction. Moderately ptilopod. No record as to brachydactyl.

No. 6. Female with three legs (*Pygopagus parasiticus*). Small, showing no indication of Asiatic origin. Neither brachydactyl nor ptilopod.

No. 8. Male. Brachydactyl, ptilopod.

Nos. 21-25. Females. Brachydactyl, ptilopod.

No. 27. Male. Brachydactyl, ptilopod.

No. 28. Male. Brachydactyl, ptilopod.

No. 29. Male. Not brachydactyl, ptilopod.

No. 30. Female. Not brachydactyl, not ptilopod.

No. 31. Female. Brachydactyl, not ptilopod.

No. 32. Female. Brachydactyl, not ptilopod.

No. 33. Female. Brachydactyl, not ptilopod.

No. 35. Female. Not brachydactyl, ptilopod.

No. 36. Male. Brachydactyl, not ptilopod.

No. 37. Male. Brachydactyl, not ptilopod.

Chicks were raised from these birds, bred together and with barred Plymouth Rocks, White Leghorns or Black Minorcas. The distribution of brachydactyl, ptilopody and syndactyl among the offspring is indicated in table I where chicks and embryos of 13 days and over are considered. (Besides those shown in the table, 305 additional chicks were produced by some of these parents after having been treated with alcohol. Of these 49 percent were brachydactyl, 37 percent ptilopod and 0 percent syndactyl. 50 percent of them showed either brachydactyl or ptilopody. These data are not included here. The effect of alcohol on the distribution of traits is discussed in another paper (DANFORTH 1919 b).

The table shows first of all a high degree of correlation between brachydactyl and ptilopody. The correlation, however, is not complete

Parental traits and mating

I. Brachydactyl ptilopod \times ne

1. Leghorn ♀ ♀ \times 8 ♂
2. Barred Plymouth Rock ♀ ♀ \times 8
3. Minorca ♀ ♀ \times 27 ♂
4. Minorca ♀ ♀ \times 28 ♂
5. 21-25 ♀ ♀ \times Minorca ♂

Total

II. Non-brachydactyl ptilopod \times

6. Minorca ♀ ♀ \times 20 ♂
7. 35 ♀ \times Minorca ♂

Total

III. Non-ptilopod brachydactyl \times

8. Minorca ♀ ♀ \times 37 ♂

IV. Non-ptilopod brachydactyl \times
ptilopod brachydactyl

9. 32 ♀ \times 37 ♂

V. Bracydactyl ptilopod \times brach
ptilopod

10. 21 ♀ \times 28 ♂

VI. Miscellaneous

11. 21-25 ♀ ♀ \times 27-28 ♂ ♂
12. 21, 30, 33. ♀ ♀ \times 36 ♂

Total

Number in each class, irrespective of

¹ One of these was syndactyl.

since some individuals are brachydactyl but not ptilopod while others are ptilopod without being brachydactyl. This immediately suggests linkage of genes and "crossing over." At the time the first paper was written this possibility could not be excluded, although it was pointed out that the fact that the two traits tend to parallel each other in their fluctuation does not favor such an interpretation.

The results of matings 6-9 seem to settle the question of two possible linked genes. In mating 6 the male was not brachydactyl but did have a very few small feathers on the tarsus and fourth toe. Mated to normal Minorca hens which did not carry determiners for either brachydactyly or ptilopody he produced both brachydactyl and ptilopod offspring. Mating No. 7 represents the reciprocal cross and yielded similar results except that, probably owing to the small numbers, no long-toed ptilopod chicks appeared. Mating 8 between a brachydactyl male with no feathers on the tarsi or toes and normal Black Minorca hens likewise yielded chicks that showed brachydactyly only, ptilopody only, brachydactyly and ptilopody combined, and the absence of both traits. Finally mating 9 in which neither parent showed ptilopody gave two chicks that were both brachydactyl and ptilopod, two that were brachydactyl only, and one that was neither.

These results seem to exclude the possibility of the different somatic combinations being due to separation of linked genes. If there are indeed two genes there is no evidence that they are ever separated since individuals showing one trait and lacking the other reproduce the missing trait as readily as those that actually have it. So far as breeding tests show, brachydactyly and ptilopody may be regarded as two manifestations of the same hereditary factor.

This interchangeability of brachydactyly and ptilopody may possibly explain some supposedly aberrant cases discussed by PUNNETT and BAILY (1918). In one case (*loc. cit.*, p. 208) a female derived from a Langshan-Hamburg cross had no feathers on the tarsi or toes but nevertheless behaved in breeding tests like a true ptilopod individual. It will be apparent that if this particular bird were brachydactyl, its behavior was entirely in accord with expectations and could not be regarded as in any way aberrant.

Similarly on p. 212, in discussing some work of BONIOTE (1914), PUNNETT and BAILEY quote a case in which two clean-legged F_1 birds from a Silky-Yokohama cross when bred together gave 8 ptilopod and 16 clean-legged offspring. If, as is highly probable, the F_1 parents were brachydactyl, the mating would be entirely comparable to mating 9 in

the table and the results are essentially the same since in both cases ptilopod and non-ptilopod young were produced. The total number of chicks in this case is so small that the relative size of the two classes has little significance especially when it is recalled that some of the 16 chicks recorded as clean-legged may nevertheless have been brachydactyl.

Likewise the data on which DAVENPORT (1909) based his statement that "two extracted clean-footed birds sometimes throw boot and sometimes not" may have come from matings in which at least one of the individuals involved was brachydactyl. In other words, if the interpretation suggested here be accepted, one of the difficulties encountered by students of ptilopody will have been removed.

The failure to recognize brachydactyly as another form of ptilopody tends in some measure to vitiate the observations thus far reported. For example, in the results from matings recorded in this paper it would make a difference of over 10 percent whether the brachydactyl non-ptilopod group were counted on one side or the other. This is a matter of considerable importance in an investigation of the genetic behavior of a trait.

The evidence for associating syndactyly with the other two traits is less conclusive but is such as to create a very strong presumption in favor of the view that such an association exists. DAVENPORT (1909) has described the trait in detail and indicated its range of variability. His data come from about one hundred and fifty individuals all descended from a single Brahma hen which was herself somewhat syndactyl. It is clear from the descriptions and figure that the progenitor of this family was also ptilopod. It is apparent too that many, possibly all, of her syndactyl descendants were likewise ptilopod. DAVENPORT's data show clearly that the trait is dominant.

Only four syndactyl chicks were obtained from the experiments reported in this paper. Two came from mating 10 and one each from matings 7 and 12. None of the parents of these four chicks was in the least syndactyl nor did syndactyly occur elsewhere in the immediate ancestry. The chicks themselves were all brachydactyl; those from matings 10 and 12 were also ptilopod. The appearance of a normally dominant trait in this manner adds some support to the suggestion that the condition found in the parent is really the same trait in a different form. Unfortunately none of these syndactyl chicks became available for breeding tests.

Another thing that strongly suggests the common origin of the three traits is the fact referred to in the paper on brachydactyly, that the same

combination of traits tends to occur in the pigeon. It is much simpler to suppose that a single mutation has occurred in one homologous gene of the pigeon and fowl than that three different genes have undergone mutation at the same time and in the same manner in these two very different species.

On the basis of the data that have now been presented the simplest and most adequate working hypothesis would seem to be that the three morphologically distinct traits, brachydactyly, syndactyly and ptilopody, owe their existence to the action of a single gene. The tendency of the trait to manifest itself in some crosses more frequently in one form, and in other crosses in another, is perhaps to be explained by the action of modifying genes unequally distributed among individuals and is in harmony with DAVENPORT'S observation that within a single manifestation (ptilopody) the grade represented by the parent shows some tendency to be reproduced in the offspring.

The characteristics and to some extent the ontogeny of these traits have been discussed by the authors to whose work reference has already been made.¹ One point, however, seems to have been entirely overlooked in previous studies of the subject, namely, the range within which the ptilopod trait varies. Ptilopody involves not only the usual presence of feathers on the tarsus but also rather fundamental changes in the scales of the foot. A normal non-ptilopod individual shows scutella arranged with great regularity over the lateral side of the tarsus and along the dorsum of the fourth toe. In the Minorca, for example, (figure 1) there are about 31 such scales in linear sequence, 17 on the toe and 14 on the tarsus. These numbers are subject to some variation but are reasonably constant. The scales themselves are smooth and regular with the distal free margins approximately straight and parallel. Only rarely is a scale divided. In ptilopod specimens (figures 2 and 3) not only are some of the scales reduced in size and thrown out of alignment but they are modified structurally, frequently showing a tendency to become raised and cylindrical. In some instances forms occur which are intermediate in character between scales and feathers. Where the reduction is carried still farther the tarsus and toe may be essentially smooth but with some of the individual scales showing longitudinal lines and grooves (figures 4 and 5). This sculptured appearance is almost always

¹ "Le vieux coq," a well known etching by the artist Felix Bracquemond, which is doubtless to be found in most museums of fine arts, portrays with remarkable fidelity all the peculiarities mentioned here except syndactyly.

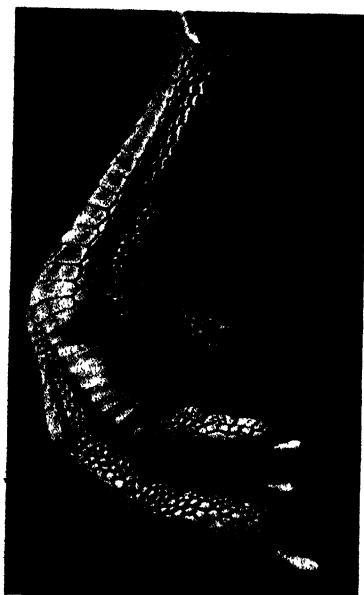


FIGURE 1.—Lateral aspect of the left foot of a Black Minorca chick showing normal proportions and the usual arrangement of scales on the antero-lateral aspect of the tarsus and on the dorsum of the fourth toe.

associated with some irregular imbrication of the scales. A series of individuals could easily be arranged to show a complete gradual transition from a condition with only a few irregular and sculptured scales to one with the heaviest degree of feathering. Indeed it might perhaps be more accurate to regard the ptilopod factor as one primarily affecting the scale-forming tissues of certain parts of the foot. In its mildest manifestation only the scales are involved. In slightly more pronounced instances there appears in addition a modified quill or rudimentary feather, generally on the basal phalanx of the fourth toe. Further intensities of manifestation may be traced through the ten stages recognized by DAVENPORT. Morphologically each feather may represent a part of the rudiment which, in the absence of the ptilopod factor, would have gone into the formation of a scale.

Despite the fact that the three traits are morphologically very different—webbing of the toes, modification of scales or feathers, and reduction in the size and number of bones are quite unlike—the possibility of their being traceable to the action of a common gene is by no means excluded. It is now commonly admitted that each gene in the germ plasm tends to affect all parts of the body more or less but that the reactions in different



FIGURE 2



FIGURE 3

FIGURE 2.—Anterior view of a (left) brachydactyl, ptilopod foot. Few of the birds referred to in the body of the paper showed a greater degree of ptilopody than this one. In addition to those on the tarsus and fourth toe a few small feathers may be seen also on the third toe. The brachydactyly which is of the more pronounced type is, as usual, most manifest in the fourth toe. This digit has only two phalanges in place of the normal five. The specimen is not syndactyl.

FIGURE 3.—Anterior view of the left foot of an individual in which the trait is present in all three forms. One or two feathers are present (ptilopody), the fourth toe is greatly reduced (brachydactyly) and the third and fourth toes are fully webbed (syndactyly).

parts may be quite diverse. With the cases in question it is easy to imagine that a disturbing factor coming into action on the 7th-8th days of incubation might be effective in producing permanent webbing of the toes. This is the time when the webbing, already present in the normal embryo, shows its most rapid involution and might be expected to be most susceptible to modifying influences. The same factor becoming effective on the 8th-10th days might be too late to have any effect on the webbing of the toes but would find the cartilages in their formative, and



FIGURE 4

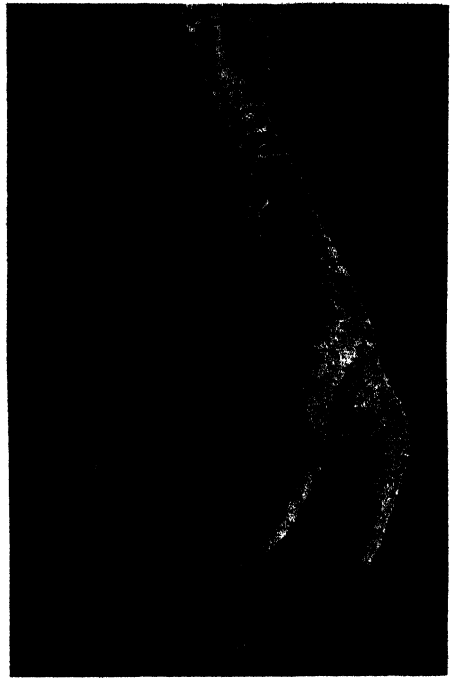


FIGURE 5

FIGURE 4.—Lateral view of a left foot showing no feathering whatever but clearly revealing the presence of the ptilopod factor by the irregularities in the scutella, some of which are "sculptured" (A). The specimen is moderately brachydactyl, the dorsal scutella being reduced from the normal 17 to about 10. The third and fourth toes are fully webbed. The falciform free margin of the web is indicated at B.

FIGURE 5.—Right foot. Similar to figure 4, but showing the sculpturing of the scales more clearly. There is a slight indication of syndactyly. Figures 4 and 5 should be compared with figure 1 in reference to the number, form and arrangement of scutella and the relative lengths of the toes.

therefore, perhaps, most susceptible stage. Finally, if not effective before the 11th-12th days, the normal webbing and skeleton of the toes would have already been determined but the possibility of feather-germ development would still remain. (For a discussion of the embryological relations of these traits the reader is referred to the paper on brachydactyly (DANFORTH 1919 a).

If one may be permitted to speculate on the mode of production of such traits several possibilities at once suggest themselves. (a) The simplest and crudest explanation suggests that the observed manifestations are the product of the action of some internal secretion upon the

normal tissues of the foot. In this case we are in reality studying the hereditary character and fluctuations in an endocrine gland through the effects produced by its secretion. (b) Conversely it might be assumed that the effect of the factor is to "sensitize" certain parts to some normal product of the endocrine system. (c) Still again, it is possible that the factor acts intrinsically and when a certain stage of development is reached the presence of a given gene in each cell of a rudiment determines the behavior of the whole mass, more or less irrespective of developments in other parts of the embryo. The problem of deciding among these and other possible alternatives belongs in the field of experimental embryology, but is necessarily of interest also to genetics.

Two interesting points raised by PUNNETT and BAILEY (1918) call for brief consideration in this connection. One of these concerns their suggestion that in the Cochin and Dark Brahma races "two factors *A* and *B* may be present, either of which determines leg-feathering," while in the Langshan and Silky only one such factor is present. The data here recorded throw no direct light on the question since no evidence appears to indicate the presence of more than one factor. But since the progenitor of these birds was a hybrid, and since the degree of ptilopody in none of his descendants was very pronounced, it is quite possible that even though the race may have been of Brahma extraction one factor had already been lost from the line of descent when the present breeding tests were begun. PUNNETT and BAILEY seem to imply that factor *A* is the more constant one, and since they offer no means of differentiating between them we may assume that their factor *A* and the gene postulated to explain the brachydactyl-syndactyl-ptilopod complex are the same. If the factor *B* is ultimately isolated in a strain of fowl it will be of considerable importance to determine whether or not it too carries the potentiality of brachydactyly and syndactyly.

The other point brought out in PUNNETT and BAILEY's report is the possible presence of an inhibitor *I*, assumed to be present in certain normal strains and tending to prevent the development of feathers, even in the presence of factor *A*. While, as previously suggested, the particular case for which the *I* factor was postulated is perhaps open to a different explanation, the possibility of such a factor being present in some cases is of interest. Such a factor introduced by the homozygous parent and coming into effect on the tenth to twelfth days might explain the very high percentage of brachydactyl non-ptilopod chicks from matings 5-9. If the existence of the *I* factor can be substantiated, a

more detailed study of its interaction with the peculiar *A* factor may yield interesting results.

To summarize briefly: The data presented in this paper show that ptilopody (the tendency to produce feathers on the tarsi and toes) is subject to a much wider range of variation than has hitherto been supposed, and that the three traits, ptilopody, syndactyly and brachydactyly, are associated in heredity. All the available evidence points strongly to the conclusion that the three traits just mentioned are primarily caused by a single gene. If the conclusions arrived at in this paper are accepted it will be necessary to revise in some measure much of the work that has been done on the heredity of ptilopody ("booting" or "leg-feathering").

The stock used for these experiments was kept in the animal quarters of the Department of Anatomy, the eggs were hatched in the laboratory incubators and the chicks raised for the most part on the roof. The writer wishes to take this opportunity of acknowledging his indebtedness to Dr. R. J. TERRY, Director of the Laboratories, for making these facilities available and for his assistance in many other ways.

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TRICOLOR INHERITANCE. IV. THE TRIPLE ALLELO-MORPHIC SERIES IN GUINEA-PIGS¹

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In a previous paper (IBSEN 1916) mention was made of a fact, at that time fairly well established by the author, that complete extension (*E*) of black or chocolate pigment, partial extension (*e^p*) of the same pigments, and non-extension (*e*) of these pigments, form an allelomorph series in guinea-pigs. No evidence for this was given at that time because the complete data were to be reserved for a later paper.

Several authors, among others notably LITTLE (1913), had given a different view as to the inheritance of these characters, and it therefore seemed advisable to obtain very complete evidence before presenting it for publication. This has now been obtained, and will be given as briefly as possible in the following pages. Since LITTLE's theory has already been discussed fully in the paper previously mentioned, it will not be taken up again here.

Before proceeding it may be well to mention that the experimental results entirely corroborate the multiple allelomorph conception. This would of itself exclude every other conception except that of complete linkage, and while these two differ from each other theoretically, they are exactly alike so far as practical results are concerned. In no single instance has a genotype been obtained which was not expected according to theory. The same, however, cannot be said with respect to phenotypes. A few animals have been born which closely resembled an unexpected phenotype, but which when tested proved to be of the expected genotype. This will be taken up later in the discussion.

The one disturbing factor is that the *proportionate numbers of individuals* in a phenotype have not always been according to expectation. This disproportion of individuals in the phenotypes, which does not in

¹ Papers from the Department of Genetics, Agricultural Experiment Station, UNIVERSITY OF WISCONSIN, No. 20. Published with the approval of the Director of the Station.

any way invalidate the allelomorphic relations of the three factors, will also be discussed more fully later.

All of the data have been placed in one large table (table 1). In a triple allelomorphic series 21 different types of mating are possible. These have all been indicated in the table, although in two cases (matings 1 and 2) no matings were actually made.

The offspring have been classified in two ways, (1) the number under each phenotype is given, and (2) those which have been tested are classified according to their genotype. Almost invariably this testing has been done by mating the animal to be tested to a self red (ee), this being the lowest in the allelomorphic series.

In each case after the recorded number of offspring a figure is placed in parentheses to indicate the expected number. The method employed in the working out of the theoretical expectation, especially in the case of the genotypes, should be explained. Mating 10 may serve as an example. Of the 87 self black offspring, 14 were tested by being mated to self reds. Of these, 12 proved to be Ee^p , while 2 were Ee . According to expectation there should have been equal numbers of each genotype, or 7 and 7. In a similar manner, of the 11 tortoises tested, 3 were homozygous ($e^p e^p$) and 8 heterozygous ($e^p e$). Since equality was expected, the number here should theoretically have been 5 of each.

We are now in a position to discuss the matings in table 1. Through mating 6 there is only one kind of phenotype, and the genotypes are close to expectation. In mating 7 we find the first example of genotypic as well as phenotypic disproportion. Instead of 3 blacks to 1 tortoise, the proportion is almost exactly 4:1 (129 self blacks:32 tortoises, and of the tested self blacks the ratio of $Ee^p:EE$ is again almost exactly 4:1, instead of the expected 2:1 (21 Ee^p :5 EE).

A further inspection of the matings shows other disproportions. In mating 8 we have an excess of tortoises instead of self blacks as in mating 7; in mating 9, a deficiency of tortoises, and in mating 13, a surplus of tortoises again. In the other matings the obtained results are fairly, and sometimes quite, close to expectation.²

What is the cause of these discrepancies? As yet none has been

²It is worthy of mention that in a previous paper (IBSEN 1916) attention was called to the fact that in $e^p e \times e^p e$ and $e^p e \times ee$ matings an excess of self reds occurred in the offspring. In the present paper, with much larger numbers of offspring in these matings, this excess has been cut down till the actual numbers are quite close to expectation. It may possibly be that with larger numbers the discrepancies in some of the crosses reported in this paper may rectify themselves.

TABLE I
All the possible crosses in the allelomorphic series.

	Mating	Offspring (phenotypes)			Tested offspring (genotypes)					
		E Self black (or chocolate)	e^p Tortoise	ee Self red	EE	Ee^p	Ee	$e^p e^p$	$e^p e$	ee
1	$EE \times EE$									
	$Ee^p \text{♀} \times EE \text{♂}$ $EE \text{♀} \times Ee^p \text{♂}$									
2	Total									
	$Ee \text{♀} \times EE \text{♂}$ $EE \text{♀} \times Ee \text{♂}$	6 49			0 (1.5)		3 (1.5)			
3	Total	55			0 (1.5)		3 (1.5)			
	$e^p e^p \text{♀} \times EE \text{♂}$ $EE \text{♀} \times e^p e^p \text{♂}$	19 0				5 (5)				
4	Total	19				5 (5)				
	$e^p e \text{♀} \times EE \text{♂}$ $EE \text{♀} \times e^p e \text{♂}$	30 6				6 (7)	8 (7)			
5	Total	36				6 (7)	8 (7)			

TABLE 1 (continued)
All the possible crosses in the allelomorphous series.

Mating	Offspring (phenotypes)			Tested offspring (genotypes)					
	<i>E</i> Self black (or chocolate)	<i>e^p</i> Tortoise	<i>ee</i> Self red	<i>EE</i>	<i>Ee^p</i>	<i>Ee</i>	<i>e^pe^p</i>	<i>e^pe</i>	<i>ee</i>
<i>ee</i> ♀ × <i>EE</i> ♂ <i>EE</i> ♀ × <i>ee</i> ♂	43 71					8 (8) 2 (2)			
Total	114					10 (10)			
7 <i>Ee^p</i> × <i>Ee^p</i>	129 (120.75)	32 (40.25)		5 (8.67)	21 (17.33)		1 (1)		
<i>Ee</i> ♀ × <i>Ee^p</i> ♂ <i>Ee^p</i> ♀ × <i>Ee</i> ♂	129 (140.25) 37 (38.25)	58 (46.75) 14 (12.75)		2 (3.3) 0 (0.7)	2 (3.3) 1 (0.7)	6 (3.3) 1 (0.7)		3 (3) 3 (3)	
Total	166 (178.5)	72 (59.5)		2 (4)	3 (4)	7 (4)		6 (6)	
<i>e^pe^p</i> ♀ × <i>Ee^p</i> ♂ <i>Ee^p</i> ♀ × <i>e^pe^p</i> ♂	18 (12) 51 (41.5)	6 (12) 32 (41.5)			1 (1) 7 (7)				
Total	69 (53.5)	38 (53.5)			8 (8)				
<i>e^pe</i> ♀ × <i>Ee^p</i> ♂ <i>Ee^p</i> ♀ × <i>e^pe</i> ♂	87 (86)	85 (86)			12 (7)	2 (7)	3 (5.5)	8 (5.5)	
Total	87 (86)	85 (86)			12 (7)	2 (7)	3 (5.5)	8 (5.5)	

TABLE 1 (continued)

All the possible crosses in the allelomorphous series.

Mating	Offspring (phenotypes)			Tested offspring (genotypes)				
	<i>E</i> Self black (or chocolate)	<i>e^p</i> Tortoise	<i>ee</i> Self red	<i>EE</i>	<i>Ee^p</i>	<i>Ee</i>	<i>e^pe^p</i>	<i>e^pe</i>
<i>ee</i> ♀ × <i>Ee^p</i> ♂ <i>ee</i> ♀ × <i>Ee^p</i> ♂	55 (50.5) 97 (106.5)	46 (50.5) 116 (106.5)				5 (5) 7 (7)		4 (4) 3 (3)
II Total	152 (157)	162 (157)				12 (12)		7 (7)
II <i>Ee</i> × <i>Ee</i>	74 (73.5)		24 (24.5)	3 (4.33)		10 (8.67)		
<i>e^pe^p</i> ♀ × <i>Ee</i> ♂ <i>Ee</i> ♀ × <i>e^pe^p</i> ♂	17 (23.5) 35 (53)	30 (23.5) 71 (53)			3 (3) 2 (2)			2 (2) 9 (9)
III Total	52 (76.5)	101 (76.5)			5 (5)			11 (11)
<i>e^pe</i> ♀ × <i>Ee</i> ♂ <i>Ee</i> ♀ × <i>e^pe</i> ♂	83 (85) 17 (17.5)	41 (42.5) 11 (8.75)	46 (42.5) 7 (8.75)		7 (7) 3 (3.5)	7 (7) 4 (3.5)		3 (3) 4 (4)
IV Total	100 (102.5)	52 (51.25)	53 (51.25)		10 (10.5)	11 (10.5)		7 (7)
<i>ee</i> ♀ × <i>Ee</i> ♂ <i>Ee</i> ♀ × <i>ee</i> ♂	71 (70.5) 111 (111.5)		70 (70.5) 112 (111.5)			5 (5) 11 (11)		
V Total	182 (182)		182 (182)			16 (16)		
VI <i>e^pe^p</i> × <i>e^pe^p</i>		36					I	

TABLE 1 (continued)

All the possible crosses in the allelomorphic series.

Mating	Offspring (phenotypes)			Tested offspring (genotypes)				
	<i>E</i> Self black (or chocolate)	<i>e^p</i> Tortoise	<i>ee</i> Self red	<i>FE</i>	<i>Ee^p</i>	<i>Ee</i>	<i>e^pe^p</i>	<i>ee</i>
<i>e^pc</i> ♀ × <i>e^pe^p</i> ♂ <i>e^pe^p</i> ♀ × <i>e^pe</i> ♂		30 (30) 21 (21)					0 (0.5) 1 (1.5)	1 (0.5) 2 (1.5)
17 Total		51 (51)					1 (2)	3 (2)
<i>e^pe^p</i> or <i>e^pe</i> ♀ × <i>e^pe^p</i> ♂ <i>e^pe^p</i> ♀ × <i>e^pe^p</i> or <i>e^pe</i> ♂	"3"	112 (115) 7 (7)					1	
17a Total		122 (122)					1	
<i>ee</i> ♀ × <i>e^pe^p</i> ♂ <i>e^pe^p</i> ♀ × <i>ee</i> ♂		128 (128) 127 (127)						6 (6) 12 (12)
18 Total		255 (255)						18(18)
19 <i>e^pe</i> × <i>e^pe</i>	"5"	133 (138)	46 (46)				6 (4.33)	7 (8.67)
<i>ee</i> ♀ × <i>e^pe</i> ♂ <i>e^pe</i> ♀ × <i>ee</i> ♂		84 (85) 181 (179.5)	86 (85) 178 (179.5)					6 (6) 7 (7)
20 Total		265 (264.5)	264 (264.5)					13 (13)
21 <i>ee</i> × <i>ee</i>		"1"	887 (888)					
Total	1246	1405	1456					

found, but a certain relation has been noticed. This relation may be stated as follows: In those matings in which both parents are self blacks or else one parent is a self black and the other a tortoise, and in which both self blacks and tortoises occur in the offspring, there is a deficiency of tortoises among the offspring when these are all homozygous (matings 7 and 9) and a surplus when they are all heterozygous (matings 8 and 13). When both classes of tortoises occur in the offspring the excess of one class (the heterozygous) tends to be offset by the deficiency of the other (the homozygous), and as a result the total number is close to expectation (mating 10).

COLE (1914, p. 350) has suggested four possible explanations for modifications of monohybrid ratios. "They might be attributed (1) to the non-viability of a particular class of the F_2 zygotes (as is the explanation offered for both the yellow mice and *Antirrhinum*); (2) to selective fertilization, i.e., to a selective union of the gametes; (3) to a disproportionate production of the two kinds of gametes; or (4) to a differential viability of the zygotes, but without the complete disappearance of any one class."

No one of these explanations can be made to fit all the aberrant cases just described. The first one, of course, does not apply at all because in none of the matings is one expected class entirely missing. The second explanation, selective fertilization, looks as if it might fit some of the matings, as for instance mating 7. Here one might say that the c^p -bearing gametes tended to unite with those carrying E . The fact that 21 of the 26 tested black offspring were Ee^p would tend to bear this out. On the other hand, if we turn to mating 9, where one of the parents is homozygous for partial extension (e^p), there is still an aberrant ratio in spite of the fact that there is no opportunity here for selective fertilization.

The third explanation, a disproportionate production of the two kinds of gametes, does not apply either, because heterozygous animals of whatever gametic composition when mated to recessive reds (ee) always produce equal numbers of the two expected classes of offspring (matings 11, 15 and 20).

The fourth explanation, the partial viability of some one class, seems to have more in its favor than either of the other three. By referring to table 2 it will be seen that in matings 7 and 9 (in which occur the homozygous tortoises) the average size of litter is comparatively high, while in matings 8 and 13 (which have the heterozygous tortoises) the aver-

TABLE 2
Average litter size for various matings.

Matings		Total number of offspring	Number of litters	Average litter size
No. 7	$Ee^p \times Ee^p$	161	52	3.10
No. 9	$Ee^p \times e^pe^p$	107	37	2.89
No. 8	$Ee^p \times Ee$	238	91	2.62
No. 13	$Ee \times e^pe^p$	153	56	2.73
No. 10	$Ee^p \times e^pe$	172	62	2.77
No. 11	$Ee^p \times ee$	314	110	2.85
No. 12	$Ee \times Ee$	98	39	2.51
No. 14	$Ee \times e^pe$	205	77	2.66
No. 15	$Ee \times ee$	364	143	2.55
No. 20	$e^pe \times ee$	529	196	2.70
Total		2341	863	2.71

age size of litter is comparatively low. From this one might infer that in the case of the last two matings there is an incomplete viability of the self blacks to account for the excess of tortoises in this mating. But even if this were the true explanation it does not account in the first two matings for the excess of self blacks and consequently the deficiency of tortoises.

When the sex ratios are examined certain aberrancies are found here also. In table 3, which has the matings arranged in the same order as in table 2, it will be found that for matings 7 and 9 the sex ratios are quite close to normal expectation,³ while in matings 8 and 13 there are marked disproportions. Disproportions occur also in some of the other matings, particularly matings 10 and 14. Why these disproportions should occur it is hard to see since the factors in this allelomorphic series are not sex-linked. Further carefully controlled experimental work is necessary in order to help clear up some of this apparent confusion.

As previously stated, some animals were born that were phenotypically contrary to expectation. This is true of matings 17 a, 19 and 21. The numbers enclosed in quotation marks in table 1 refer to these animals. In the first two of the above-mentioned matings some apparently self-black animals were born from tortoise parents. It had been noticed in a number of cases that animals which were apparently self black at birth later showed a few red hairs and so were undoubtedly tortoises and

³ The ratio for the total 2341 animals in the table is 105.93 males to 100 females. This approximates that found in many other animals.

TABLE 3
Sex ratios for various matings.

Matings	Sexes of offspring						Unclassified offspring			Total offspring
	E		e ^p		ee		E	e ^p	ee	
	♂	♀	♂	♀	♂	♀				
No. 7 Ee ^p × Ee ^p	67	62	19	13						161
No. 9 Ee ^p × e ^p e ^p	36	33	18	20						107
No. 8 Ee ^p × Ee	91	74	29	43			1			238
No. 13 Ee × e ^p e ^p	38	14	49	49				3		153
No. 10 Ee ^p × e ^p e	42	45	34	50				1		172
No. 11 Ee ^p × ee	77	73	77	82			2	3		314
No. 12 Ee × Ee	38	34			11	11	2		2	98
No. 14 Ee × e ^p e	41	54	25	25	31	20	5	2	2	205
No. 15 Ee × ee	93	81			94	79	8		9	364
No. 20 e ^p e × ee			137	120	131	130		8	3	529
Total	523	470	388	402	267	240	18	17	16	2341

were classified as such. Some of the animals listed as self blacks were born dead and hence had to stay classified as such. The few, however, that remained self black in appearance when adult, behaved as tortoises when mated to self reds (*ee*) or tortoises. It is probable that few if any "selves" or almost selves would have been produced were it not for the fact that selection was being practiced in a plus direction in a definite attempt to produce actual *E* selves in this manner. So far, as above indicated, the attempt has been entirely unsuccessful.

In mating 21 we have another example of an apparent dominant being produced from two recessives. Here, what looked like a tortoise was born from self red parents. The animal in question, A 85.1, was entirely red except for a very small chocolate patch back of the left ear. It was one of a litter of five all of which unfortunately died shortly after birth. The parents have had 14 offspring altogether, and of these 13 were self reds.

The aberrant "tortoise" might be looked upon as a mutation, but evidence based on another animal indicates that it was genetically a self red (*ee*) in which the non-extension factor (*ee*) was "accidentally" over-expressed. The other animal referred to is 665.2, which was red with a few small white patches on the head and a very small black area in front of the right ear. She came from an *e^pe* × *ee* mating and was originally classified as a tortoise (*e^pe*) (IBSEN 1916, p. 302). However, when

mated to self reds she had 15 offspring, all self reds. Tortoises as a rule have at least half of the body surface covered with black patches. The two animals, A 85.1 and 665.2, referred to above, had far less black (or chocolate) pigmentation than any genotypic tortoise so far born in our laboratory, and therefore in spite of the black patches on their bodies must be looked upon as genetically self reds.

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